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Volume 41 September, 1951 Number 9

Phytopathology

AN INTERNATIONAL JOURNAL

Official Organ of

The American Phytopathological Society

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Subscription price: $10.00 per year, domestic and foreign

Office of Publication

Corner North Queen Street and McGovern Avenue, Lancaster, Pennsylvania

Entered at the postoffice in Lancaster, Pennsylvania, as second-class matter. Acceptance for mailing at the special rate of postage provided for in paragraph (d-2) Section 34.40, P. L. & R. of 1948. Authorized May 12, 1921.

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THE FEEDING SEQUENCE OF PSEUDOCOCCUS BREVIPES (CKL) IN RELATION TO MEALYBUG WILT OF PINEAPPLES IN HAWAII[1]

WALTER CARTER

(Accepted for publication April 2, 1951)

The basic hypothesis of the nature of mealybug wilt is that it is caused by a toxic secretion produced by the insect when it feeds in relatively large numbers and for a considerable time on pineapple plants. According to this hypothesis in its simplest form mealybugs move into the fields from wild hosts on the margins, feed on pineapple plants, and then move, often en masse, to contiguous pineapple plants. Once this process begins, "edge wilt" creeps in from the margins of fields until sometimes entire fields are involved and great losses ensue (1, 2, 3).

This hypothesis has been the basis for controlling wilt by reducing mealybug populations established on plants to a level below which wilt would not ensue. Control has been improved by still further reduction in the incidence of large colonies of mealybugs; lapses, by the converse process.

An experimental difficulty, however, has been the lack of consistency in results even when gross infestations, numbering several hundred insects of all sizes, were used on single plants. This study, begun in 1942, therefore aimed at clarification of this phenomenon, and the problem has been approached by experimental variation of the host-plant sequence, each sequence ending in a transfer of the insects to a test plant.

WILT RESULTING FROM TRANSFER OF MEALYBUGS FROM INFESTED PINEAPPLE FIELDS TO HEALTHY UNINFESTED TEST PLANTS

The transfer of mealybugs from infested pineapple plants growing in plantation fields to test plants grown in experimental plots is the basic feeding sequence of these experiments.

Wilt resulting from these transfers served as a check on the mealybugs used from the same sources for experimental host sequences, and for satisfactory comparisons it was desirable that the check infestations should result in a high percentage of wilt. Actually, this occurred with disappointingly low frequency, and many tests were inconclusive because of a low percentage or absence of wilt in the infested check plots. The technique of collection has been standard for many years: it is the removal of mealybugs from field-collected fruits and separation of the mass of mealybugs into aliquot samples. Immature fruits are collected from wilting areas in ratoon fields. Since mealybug colonies on wilted plants are normally too small to be useful, collections are made from apparently normal plants growing among wilted ones. No great uniformity in relative numbers of wilted and healthy

[1] Published with the approval of the Director as Technical Paper No. 199 of the Pineapple Research Institute of Hawaii.

plants in an area can be found, so that collections from different fields differ in that respect. However, in this series the collection of infested fruits from wilting areas was a denominator common to all the collections.

In all the tests, summarized in table 1, infestation was with 100 medium-sized mealybugs per plant, and usually 10 plants were infested in each test.

During the 6 years of these experiments, it was evident that collections from some pineapple-growing localities were more effective in inducing wilt than were collections from other areas, and the final summarization of the data showed this to be true (Table 1). Area A, which includes the least fertile and most worn and eroded fields, was the most effective source of wilt-inducing mealybugs, although even in this area the percentage of wilt varied from none in one test to 100 per cent in another, but with 13 out of 18 tests showing 50 per cent or more wilt. Area B is a dry area but a high producer with adequate rainfall. While areas C and D are separated, they are roughly equal in characteristics except that area D includes more un-

TABLE 1.—*Results from 73 tests involving direct transfer of mealybugs from field-grown pineapple plants to test plants*

Area	Number of tests	Number of tests in each percentage wilt class										
		0	10	20	30	40	50	60	70	80	90	100
A	18	1	0	2	2	0	2	2	3	1	4	1
B	10	4	2	1	0	1	0	2				
C	9	4	2	1	0	1	0	1				
D	28	9	2	3	7	4	0	1	1	0	1	
E	8	1	1	4	0	2						
Total	73	19	7	11	9	8	2	6	4	1	5	1

favorable soil types and has a somewhat greater rainfall favoring some soil pathogens. Areas B, C, and D all showed completely negative results in a high percentage of cases, with 50 per cent or more wilt occurring only occasionally. Area E is the most fertile and otherwise suitable pineapple growing section of the five areas studied; mealybugs from this area were the least effective in inducing wilt.

WILT RESULTING FROM DIRECT TRANSFER OF MEALYBUGS FROM WILT HOSTS TO PINEAPPLE PLANTS

In the writer's original paper on mealybug wilt, a low percentage of positive cases was recorded with mealybugs from natal red-top grass (*Tricholaena repens* (Willd.) Hitchc.) and panicum grass (*Panicum purpurascens* Raddi).

It has not been possible to repeat these results, and no direct transfer from host plants other than pineapple has resulted in wilt in the later tests reported here, which covered a period of several years.

This series of experiments included both green-spotting and non-green-spotting strains of *Pseudococcus brevipes* collected from host plants other

than pineapple and growing in waste places or on headlands. Collections from leaves of sisal (*Agave sisalana* (Engelm.) Perrine) and yucca (*Yucca glorisa* L.) were of green-spotting strain. Those from the grasses, celery (*Apium graveolens* L. var. *dulce* DC), and taro (*Colocasia esculenta* (L.) Schott) were customarily from the subsurface portions of the stems or the roots and were of the non-green-spotting strain.

Nineteen tests were made, using 10 to 450 mealybugs on each plant and from 10 to 36 plants in each test, from sisal, yucca, celery, taro, wire grass (*Eleusine indica* (L.) Gaertn.), sandbur (*Cenchrus echinatus* L.), panicum grass, and natal red-top grass. All of these tests were negative, except one mild case developing out of 21 plants infested with mealybugs from sandbur. The only other symptoms were a slight reddening of the leaves and, in a few cases where the larger numbers of mealybugs were used, a tendency for the leaves to droop in rosette fashion. Both these symptoms were transitory and did not progress to typical wilt symptoms.

TABLE 2.—*Tests with mealybugs collected from yucca and fed on pineapple plants before transfer to test pineapple plants*

Test[a]	Intermediate host	Test plants	
		Infested	Wilted
1	Cayenne clone LH20	10	1
	Do. L100	10	0
	Do. M4W	10	0
	Do. M4W	5	1
	Resistant hybrid 10572	2	2
	Resistant hybrid 7898	1	1
2	Caged fruiting seedling	8	0
3	Caged fruiting seedling	21	0
4	Fruits of resistant hybrid 7898	20	20
5	Resistant hybrid 7898	15	5

[a] Gross masses of mealybugs were used in tests 1 through 4, but 25 mealybugs were used in test 5.

WILT RESULTING WHEN MEALYBUGS FROM NEGATIVE HOSTS WERE FED ON PINEAPPLE PLANTS BEFORE TRANSFER TO TEST PINEAPPLE PLANTS

In these tests, mealybugs from yucca, which have proved entirely negative in all the tests thus far, were caged on pineapple plants before transfer to test pineapple plants. In some cases, notably when the intermediate feeding was on resistant varieties, positive results were obtained, indicating that during the intermediate feeding the insects became capable of inducing wilt of the next pineapple plant fed upon. The results are in table 2.

THE DETACHED PINEAPPLE LEAF AS A SOURCE

The growth habit of the pineapple plant and the normal feeding position of the mealybugs deep in the center of the plant make it essential to dissect the plant in order to collect enough mealybugs for infestation purposes. Even with heavily infested fruit it is not practical to remove the mealybugs except from the detached fruit.

Maintaining colonies of *P. brevipes* on specific plants is therefore difficult, and maintaining these colonies on caged plants even less satisfactory, first, because the colonies do not flourish for long without ant attendance and second, because plants grown under shaded conditions in cages have proved negative in many previous attempts.

Pineapple plants, however, are tolerant of a certain amount of mechanical leaf loss. Leaves that are not fully mature are easily pulled out, and

TABLE 3.—*Results from experiments in which whole leaves were compared with leaf sections as source material*

Source leaf	Cage[a]	Wilt stage	Source leaf	Cage[a]	Wilt stage	Source leaf	Cage[a]	Wilt stage
Whole leaves								
9B	A	2	32C	A	2	33A	A	4
	B	4		B	2		B	4
	C	2		C	0		C	0
	D	4		D	3		D	0
	E	0		E	0		E	1
26A	A	1	4A	A	4	33C	A	3
	B	3		B	4		B	1
	C	4		C	0		C	3
	D	4		D	3		D	4
23B	A	0	4B	A	Tips only			
	B	0						
	C	4		B	3			
	D	4		C	1			
Cut leaves								
3A	A	4	20A	A	0	30B	A	4
	B	4		B	0		B	1
	C	4		C	3		C	4
	D	0		D	2		D	4
	E	0		E	4		E	2
16A	A	0	26D	A	0	33B	A	1[b]
	B	1[b]		B	4		B	0
	C	0		C	2		C	4
	D	0		D	2		D	4
	E	0						
22A	A	0	12B	A	2			
	B	4		B	3			
	C	3		C	4			
	D	4						

[a] Cages are lettered from the upper green portion, A, to the green-white tissue, C to E, depending on the length of the leaf.
[b] Dubious cases.

if set base down in water will retain turgidity for a long time. This makes it possible to conduct feeding tests without injury to the plant caused by the feeding of a large mealybug colony on it. The technique required a feeding cage shaped to the normal curvature of a pineapple leaf, and in this development Dr. Carl T. Schmidt was of material assistance. Such a cage has been standard for some time as an essential part of feeding-sequence experiments involving pineapple plants.

The experiments described below were conducted to determine the most suitable section of the leaf, if any, to which the cage should be attached. The normal feeding position for mealybugs is on the lower portion of the leaf in the pale-green tissue above the basal white tissue, where, in a test plant, mealybugs must feed if wilt is to occur. Feeding of mealybugs on the upper darker-green portions of a growing test plant has failed to result in wilt, except in a few cases where very large numbers of mealybugs were used.

It was of interest therefore to learn whether this localized feeding was also necessary to establish a plant as a source of mealybugs capable of inducing wilt.

Leaves were pulled from field-grown plants of the wilt-resistant hybrid No. 7898 and divided into two lots. The leaves in one lot were kept intact, and mealybug cages attached about 2 inches apart over the whole length. The leaves of the second lot were cut into pieces long enough to accommodate one cage and allow enough leaf below the cage to permit placing it in water in a shallow dish.

Mealybug infestations were of two kinds: in one series, 100 medium-sized mealybugs were used; in another, 10 gravid females. In the latter case, the crawler colonies were transferred. The results of this experiment (Table 3) indicate clearly that there was essentially no difference between whole leaves and leaf sections as source material.

None of the leaves was entirely negative, although one (16A) showed only one questionable positive. Five leaves were positive throughout. There was no consistency in the position of the negative cases, some being from distal cages, some from proximal.

Variation in symptom expression in test plants infested from the same source leaf is of interest, since from a single source leaf the entire symptom range was obtained.

EFFECT OF ARTIFICIAL FEEDING OF MEALYBUGS ON THEIR ABILITY TO INDUCE WILT OF PINEAPPLES

In these experiments, the mealybugs were removed from field-collected pineapple fruits and fed on agar plates (4). Usually the agar plates were made up with a simple 3 per cent agar without additives or pH control. When a nutrient agar was used, it consisted of 3 per cent dextrose and 0.5 per cent peptone. In the summary of these experiments, which follows, modifications of the agar are noted.

After feeding for a varying number of days, the mealybugs were transferred to test plants in the field. As checks on these experiments, aliquot samples of mealybugs were taken directly from the gross colonies collected from the field, and test plants were infested with them directly without an intervening period of artificial feeding. All experiments in this series in which no wilt resulted from the check infestations have been omitted from table 4.

The number of mealybugs used in all these tests was 100 per plant for both artificially fed mealybugs and check infestations.

While the majority of these experiments showed negative results following artificial feeding, some positive results were obtained; and those following 4, 2, and 5 and 3 days' feeding in tests 2, 9, 10, and 11, respectively, are

TABLE 4.—*Effect of artificial feeding on the ability of mealybugs to induce mealybug wilt*

Test	Number of days on agar	Number of plants		Modification of feeding medium
		Tested	Wilted	
1	6	5	0	Tap water: pH 7
	6	7	0	1/3000 Riboflavin
	0	10	3	.....
2	4	10	4	Tap water: pH 4
	4	10	1	Ascorbic acid 1 per cent
	0	10	5	.....
3	7	17	0	Tap water at pH 5 + 3 per cent dextrose; 5 per cent peptone
	7	12	0	Nutrient agar + 1 per cent ascorbic acid
	0	17	10	.....
4	6	18	0	Nutrient agar
	6	23	1	Nutrient agar + 100 mg./litre biotin
	0	16	14	.....
5	3	17	0	Nutrient agar
	3	16	0	Nutrient agar + pineapple leaf juice
	0	10	4	.....
6	6	8	0	Tap water: pH 7
	6	4	0	1: 1000 Alpha naphthaleneacetic acid
	0	10	9	.....
7	3	10	0	Tap water: pH 4
	3	10	0	Tap water: pH 2.6
	0	10	5	.....
8	5	16	0	Tap water: pH 2.6
	5	10	0	Tap water: pH 4
	0	10	3	.....
9	2	10	4	Tap water: pH 7
	2	1	1	Tomato juice agar
	0	10	7	.....
	6	10	0	Tap water: pH 7
10	5	13	0	Nutrient agar
	5	10	3	Tomato juice agar
11	3	7	1	Tomato juice agar
	3	7	2	Tomato juice agar
	0	10	2	.....

undoubtedly significant. It is clearly indicated also that tomato juice in the feeding medium has some effect.

THE SEEDLING PINEAPPLE AS A SOURCE PLANT

In these tests, source plants were pineapple seedlings, crosses between pineapple varieties and variety hybrids. Seedlings were grown until approximately 6 months old in greenhouse and lath-house and then transplanted to the field. Some of the tests were made with these plants shortly after their removal from the lath-house to full sunlight, others after the

plants had been well established following transplanting to the field. In view of the mealybug control maintained on these seedlings, they can be considered as having no previous history of mealybug feeding.

The procedure followed was to pull leaves from these seedlings, feed mealybugs on the detached leaves, and later transfer the infested leaf sections to test plants.

Test 1 of this series used leaves from two seedling lots growing in the field. One hundred medium-sized mealybugs were caged on the median green tissue and 5 gravid females were caged on the white basal tissue of each leaf. After 11 days feeding, the colonies of mealybugs which had developed were transferred to test plants. One series of mealybugs was transferred to LH20, a susceptible Cayenne clone; another series was transferred to other plants of the same seedling clones from which the source plants were taken. The results were entirely negative.

In another experiment of this type, plants from three separate seedling clones were used as source plants. Again, gravid females from yucca were

TABLE 5.—*Tests of Cayenne Clones as source plants*

Clone	Number of second-generation			
	Plants		Slips	
	Tested	Positive	Tested	Positive
FR (field-run selected at random)	30	1	28	2
LH8	30	1	34	3
LH20 (very susceptible)	30	3	27	0
LH69	30	1	30	0
No. 573 (resistant)	30	11	23	18
No. 666 (resistant)	30	5	13	12

caged on detached leaves of these seedlings, and 13 days later the colonies of mealybug crawlers which had developed were transferred to field-run Cayenne test plants. Again, no typical wilt developed.

A third experiment, using three seedling clones as source plants, was done in the same summer, the test plants being field-run Cayenne and other plants of the same seedling clones as were used for source feedings. This experiment was also entirely negative.

In these three experiments, 80 plants from eight separate seedling lots were used as source plants. The entirely negative results are of considerable interest since they indicate clearly that the seedling pineapple plants used, with no previous record of mealybug feeding, were not positive source plants for mealybug wilt.

CAYENNE CLONES AS SOURCE PLANTS

A planting of Cayenne clones had been maintained en bloc for two generations. During this time mealybugs were carefully controlled, and no wilt occurred. During the growth of the second generation, some of these

plants were tested by the detached-leaf technique. When the second-generation plants were mature and vegetative slips were removed, a leaf was pulled from one slip from each of 155 mother plants and tested as a source plant. The results are in table 5.

The five positive slips from Clones FR and LH8 were from mother plants which had been negative in earlier tests. Ten of the 18 positive slips from Clone 573 had been tested previously and only four had been positive. The 12 positives of Clone 666 in the slip tests included eight slips tested earlier, but only three of the eight had been positive in the first test.

Thus, consistently positive vegetatively propagated source plants are relatively rare. Clone 666, which is perhaps the most positive source plant of the Cayenne clones, is nevertheless not consistent, as shown by the following experiment.

Leaves were pulled from 20 plants of Clone 666, gravid mealybugs were fed on these leaves for 12 days, and the crawler colonies were transferred to the susceptible Clone LH20. Twelve wilted. The experiment was repeated 2½ months later with 10 of the plants positive in the first test as source plants. Only three of these proved positive in the second test.

A confusing factor is the variation in susceptibility of test plants, even of the most susceptible clone. A single planting may be very susceptible in many tests but a single test may be negative. For example, 10 plants in each of four plots of Clone LH20 were infested with crawler colonies, grown respectively on Clones LH20, 573, 666, and the resistant hybrid 10572. Not a single plant wilted, although three of these four source clones are normally good sources. In the same plots other tests immediately before and after this test were positive.

FEEDING SEQUENCES INVOLVING A RESISTANT VARIETY PRIOR TO TEST FEEDING

Most important was the discovery that resistant hybrid 7898 was a fruitful source plant for the development of toxicity in mealybugs. For the first time it was possible to eliminate at least some of the variability in results described in the previous section (5). The results from 15 tests with this hybrid are in table 6. Mealybugs were from field-collected pineapple fruits.

Although the resistant variety 7898 is a much more reliable source plant than is field-run smooth Cayenne, there is nevertheless considerable variation in results even when large numbers of insects are used.

Test 8 in part and test 10 were negative with only four test plants in each. Test 12, using varying numbers of insects, indicates that even with 7898 as a source plant, increasing insect numbers increases the possibility that test plants will wilt. In test 14, transfers from 7898 grown in water vapor culture induced less wilt than transfers from potted plants. However, all but two of the colonies of mealybugs transferred to these test plants were small, comprising less than 10 bugs each. Two colonies were of fair size, and one of these wilted the test plant.

Open and caged pot cultures were compared to determine the feasibility of maintaining colonies of mealybugs on caged plants of 7898. Slips were planted in a 5-gal. container, left in the open and uncovered. Sister slips were planted in similar containers but covered with cloth and celluloid cages.

Three months after planting, mealybugs were collected from 7898 in the field, 10 lots of 100 medium-sized bugs were transferred to field-test plants directly and lots of 10 gravid females were caged on each plant. Cages attached about half way up the longest leaf were left on the growing plants for 25 days and then cut off, and leaf sections were transferred to test plants in the field.

TABLE 6.—*Wilt resulting when mealybugs were fed on resistant variety 7898 and transferred to test plants*

Test	No. of mealybugs used	No. of test plants		Notes on source feeding on 7898
		Infested	Wilted	
1	100	10	8	Bugs starved overnight after source feeding.
2	100	10	8	Bugs not starved.
3	100	10	8	Direct from field-grown plants.
	100	10	6	Fed for 27 days on detached crowns.
4	Gross	8	5	Fed on green fruits 3 months.
5	Gross	20	20	Fed on green fruits 6 weeks.
6	Gross	8	4	Fed on crowns 1 month.
7	100	10	9	Caged 6 days on field-grown plants.
8	100	7	7	Fed on detached leaves 6 days.
	100	4	0	Fed on slip 9 weeks.
9	200	7	5	Fed 5 days on field-grown plants.
10	Gross	4	0	On green fruits for 14 days.
11	15–35	10	10	Direct from field-grown plants.
12	10	10	1	Direct from field-grown plants.
	20	5	3	do
	50	5	3	do
	100	10	8	do
	100	10	10	do
13	Gross	16	16	Crawler colony fed for 11 days.
14	Gross	10	7	Crawler colony on potted plants.
	Gross	20	1	Crawler colony on detached leaves of plants grown in vapor culture.
15	100	10	5	Caged on field-grown plants 9 days.
	16–61	10	10	Bred on same plants.

The following day the same two lots of source plants were reinfested with 100 medium-sized bugs from 7898 growing in another field. Ten test plants in the field, and 1 week later a second lot of 10 plants, were infested directly. Cages were cut off 13 days after they were placed on the source plants, and leaf sections were transferred to field-test plants. The mealybugs transferred from 7898 to the field induced 100 per cent mealybug wilt in both cases. From the 7898 growing in the open in cans there were seven cases out of 11 in the first transfer and 10 out of 10 in the second. Mealybugs fed on caged plants, however, induced wilt in only 1 of 10 plants infested in the two series.

A new series of plants was set out on January 5 in a similar manner, except that in this case slips were not available and mature suckers were

used; by April 29 most of them had prematurely fruited. The small crowns were cut from the immature fruits, and 100 mealybugs applied to the center of each crown, after which the crowns were set base down in beakers covered with light cloth tops. The results of this test were not in agreement with the results of the tests mentioned above. The shaded fruiting plants proved as positive as those growing in the open cans. There is strong indication herein that the physiological state of a normally positive clone can affect the status of that clone as a source plant.

WILT RESULTING WHEN MEALYBUGS FROM A RESISTANT VARIETY WERE TRANSFERRED TO TEST PLANTS AFTER AN INTERMEDIATE FEEDING ON CAYENNE CLONES

These experiments were the converse of those described in the preceding series. Mealybugs were transferred from 7898 to selected Cayenne clones, fed for 10 to 14 days, and then transferred to field-grown test plants. It is

TABLE 7.—*Mealybugs from 7898 transferred to test plants after first feeding them on selected Cayenne clones*

Test	Source clone	Mealybugs used	Number of test plants	
			Infested	Wilted
1	LH20	Gross	10	0
	L100	do	10	0
	M4W	do	10	1
2	666	100	6	2
	666	100	6	6
	LH20	100	6	2
	LH20	100	6	1

evident from table 7 that, except for one transfer from a resistant Cayenne clone (666), the capacity to induce wilt was either very much reduced or lost entirely.

DISCUSSION

When wilt incidence from random collections of mealybugs is considered, it is noted that the largest class in the distribution, 19 cases of 73, is the negative one. All these tests used 100 mealybugs to each test plant.

It is clear from this, that field-run pineapple plants are not, on the whole, good sources of mealybug wilt. This is remarkable in view of the fact that there has been no reintroduction of pineapple plants for commercial use in Hawaii for 50 years. Furthermore, up to 1932 the relationship of mealybugs to pineapple wilt was not fully understood, and no control was known. At that time, crop failure, partial or complete, in ratoon crops was the rule rather than the exception. Control was achieved in the years following; and at the outbreak of war in 1941, wilt on any but an incidental scale was rare. There was then a gradual deterioration of control efficiency during the war years, and by the end of the war this situation, accentuated by

drought which limited the use of water, had culminated in widespread wilt on many plantations. Mealybugs had again become widely distributed, their populations were high, and pineapple planting material was heavily infested.

The history of mealybug infestation and accompanying wilt, coupled with the fact that the current pineapple plantings have been vegetatively reproduced for 50 years, would lead to the expectation that all field-run Cayenne plants in the Hawaiian area would be positive sources of mealybug wilt if the disease were infectious and if it were transmitted through vegetative planting pieces. The evidence is clearly contrary, unless some unusual type of latency is postulated.

On the other hand, there is an indication in the data that field-run Cayenne as a source of mealybug wilt is conditioned by differences in the synecological factors of soil, agronomy, and weather, in localities 3 or 4 miles apart. This would not be surprising in view of the growth differences commonly noted between fields.

There are complicating factors, notably the transfer of planting material from one location to another, and the carry-over of vegetative strength or weakness to the new generation. The question is being explored experimentally by means of sister slips of many clones grown in six separate locations and used as source material. Such an experiment requires a minimum of 3 years for completion; not until fruiting of the second crop are the data available comparable with those used in table 1.

Experiments using detached leaves, either whole or cut into sections, show that the mature green portions of the leaf are as suitable as sources as are the proximal, lighter-green tissues. However, mealybugs must feed on the latter tissue of test plants before wilt results.

The last feeding prior to transfer to test plants is determinative; feeding on agar results in loss of wilt-inducing capacity, but the effect of tomato-juice diet is suggestive of some nutritional influence, even though the material is too complex to be satisfactory experimentally.

Failure of seedling pineapple plants as sources of mealybug wilt is difficult to account for if the toxin hypothesis is to be based on a simple pineapple-to-pineapple sequence. Granted that many of these seedlings differ from Cayenne in many respects, some at least should prove positive as sources, unless this capacity is related to genetic resistance. In the latter case, very large seedling populations would require study.

The instability of Cayenne clones as source plants is shown in table 5. This instability is also demonstrated when the most positive source—the resistant variety and clone—is used. There is evidence also that environmental conditions modify the plant as a source. However, the most positive and conclusive evidence is that the resistant variety and resistant clones of Cayenne are the best and most consistent sources, and in current tests the gross colony from field plantings is rarely used, this method having been replaced by the detached-leaf technique applied to the resistant type.

Finally, the transfer of mealybugs from a good source plant to an intermediate feeding on poor source plants prior to test plant infestation results in the loss of ability to induce wilt.

SUMMARY

When mealybugs from infested fruits growing among wilted pineapple plants were transferred to test plants, a large percentage of such tests were completely negative. In only a relatively small percentage of tests did more than 50 per cent wilt occur.

An area in Hawaii which includes the least fertile and most worn and eroded fields was the best source of wilt-inducing mealybugs.

Sections cut from detached pineapple leaves can be used as a source by feeding test mealybugs in a special cage. The entire wilt-symptom range has been obtained on a series of test plants infested with mealybugs from a single leaf.

Artificial feeding of mealybugs usually resulted in elimination of wilt-inducing capacity, but in three of four exceptions the mealybugs had been fed on tomato-juice agar. Mealybugs transferred from wild hosts to pineapple test plants gave negative results throughout, these results being at variance with earlier published data.

Selected Cayenne clones were tested in two generations to determine the consistency of these vegetatively propagated plants as wilt sources. The most consistent sources were Cayenne clones known to be resistant to mealybug wilt. Even these showed considerable variation. Seedling pineapple plants without known history of mealybug infestation were negative as sources. The resistant hybrid 7898 has been confirmed as a positive source plant, but environmental conditions, such as deep shade, may affect this adversely. Mealybugs from 7898, given an intermediate feeding on Cayenne clones, were rendered less toxic except when the resistant Cayenne clones were used.

Mealybugs from the negative host, yucca, when given an intermediate feeding on resistant Cayenne clones and varieties, induced high percentages of wilt of the test plants.

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BUD TRANSMISSION OF VERTICILLIUM IN ROSES

A. W. Dimock

(Accepted for publication April 3, 1951)

The Verticillium disease of roses, which is characterized by yellowing and abscission of leaves and die-back of invaded canes, has recently been brought into focus by a number of cases of loss in greenhouse roses, some serious and some of lesser importance, and several instances in garden roses. An apparent increase in incidence of the disease may be due in part to correct diagnosis through better knowledge of symptoms. A real increase, however, may have resulted from the recent rather general shift from the practice of grafting the flowering variety onto imported *Rosa manetti* understocks at the individual grower's range to that of buying plants already budded to the flowering variety on *manetti* understocks grown on the Pacific Coast.

In the majority of the cases studied the evidence strongly suggested that the pathogen was introduced with the plants and was not acquired from infested soil in the greenhouse beds. Numerous infected plants often were observed in individual shipments from the Pacific Coast while none were found in other shipments of the same varieties planted in the same soil. In cases of light disease incidence the infected plants were distributed at random throughout the beds. Little or no definite evidence of spread of the pathogen from infected plants to adjacent healthy plants was observed. Suggestive also was the fact that in almost every instance the disease developed within a relatively few months after planting; it was not observed in older established plantings. Furthermore, nearly all cases observed were in concrete-bottom beds in which the soil had been thoroughly steamed prior to planting.

The most convincing evidence that the disease was being introduced with the plants was the fact that *Verticillium albo-atrum* R. & B. was readily isolated from the xylem tissues of the flowering wood but not from the *manetti* rootstock. The apparently high resistance of *manetti* rootstocks would preclude sufficiently rapid development of infection from the soil of the greenhouse beds to account for the high incidence of the disease encountered in some of the young plantings.

Analysis of the cultural operations involved in the production of budded plants suggested two possible mechanisms of inoculation. First, inoculum from infested soil might enter the wound made at the time of budding. Second, Verticillium-invaded buds taken from infected canes of the flowering variety would obviously serve to perpetuate the disease if the bud "took," and might serve to successfully inoculate a susceptible understock even though the first budding failed. Proof of the possibility of bud transmission is here reported.

EXPERIMENTAL EVIDENCE

Preliminary to actual bud-transmission tests, bud slivers from a number of rose canes with typical symptoms of Verticillium disease were plated out and in most cases *Verticillium* was recovered. Typical was one instance involving 13 consecutive buds from a single cane: nine yielded *Verticillium*, three were negative, and one became contaminated. Of the nine positive buds, six were brown and apparently dead, but three were green and subtended by apparently normal leaves.

In the first of two series of bud-transmission tests, eleven plants of the variety Edith Willkie were budded December 3, 1948, with buds taken from canes showing symptoms on Better Times plants which had been inoculated with *Verticillium* at a point just above the soil line on October 31. Three other Edith Willkie plants were wound-inoculated with a rose isolate of *Verticillium*, and two were budded with buds from a healthy Edith Willkie plant. Symptom expression, if any, was recorded, and on March 30, 1949, isolations were made (a) from the budded Edith Willkie canes at a point 2 in. or more above the bud, (b) from a second shoot originating below the bud or point of inoculation when such a shoot was present, (c) from the main stem at the soil line, and (d) from the roots. In several cases where a bloom developed on the budded shoot, isolations were made from the flower stem when cut.

Of the eleven plants in which diseased buds were inserted, eight developed definite symptoms and *Verticillium* was isolated from five of these. Neither of the two plants budded with healthy buds, and none of the three plants which were budded with diseased buds and which failed to show symptoms, yielded *Verticillium*. It is significant, however, that *Verticillium* was isolated from flower stems before symptoms were evident on two of the positive plants budded with diseased buds. Symptoms developed and *Verticillium* was reisolated from all three culture-inoculated plants.

In the second series of tests, 20 young own-root Better Times and 20 budded Briarcliff on *manetti* understock were employed. On January 1, 1949, ten of each were budded with buds from diseased Better Times canes from inoculated plants; five were inoculated with *Verticillium* from culture; and five were budded with healthy Better Times buds. Data were taken as described for the Edith Willkie plants. On April 1, five of the ten Better Times plants budded with diseased buds had definite symptoms, and from three of these *Verticillium* was isolated from the budded stem. Three of the five culture-inoculated Better Times had definite symptoms and yielded *Verticillium* from the inoculated stem. Two of the five plants budded with healthy buds had questionable symptoms but neither of these, nor any of those without symptoms, yielded *Verticillium*.

Of the ten Briarcliff plants budded with diseased buds, five had definite and one had questionable symptoms on April 1. *Verticillium* was isolated from the budded stems of two of the definite plants and from the question-

able one. Of the five culture-inoculated plants two developed definite symptoms, and two developed questionable symptoms but yielded *Verticillium*. All other plants failed to show symptoms or yield *Verticillium*.

In summary, infection was definitely established and confirmed by isolation in at least 11, or 35 per cent, of the 31 plants budded with buds taken from Verticillium-infected rose canes, and in eight, or 62 per cent, of the 13 plants inoculated with *Verticillium* from agar cultures. Including the cases in which symptoms were definite but isolation attempts unsuccessful, the percentages become 61 and 77, respectively. None of the 12 plants budded with healthy buds became infected. Significant also is the fact that when 3 to 4 months had elapsed after inoculation, *Verticillium* was in no case isolated from the roots, the base of the main stem, or other branches originating below either the diseased buds or points of culture-inoculation,[1] although it was readily recovered from points a foot or more up on the inoculated stems. In the majority of cases the inserted buds died; a few "took" but failed to "break"; one took, and formed a new shoot even though it had served to transmit *Verticillium* to the previously healthy Edith Willkie. Failure of the buds was probably due largely to admittedly poor technique, since the mortality was as great in the healthy ones as in those from the diseased canes.

DISCUSSION

These tests show unquestionably that buds taken from Verticillium-infected rose plants may serve to successfully inoculate the canes of susceptible varieties into which they are inserted. With susceptible understocks this undoubtedly could serve to transmit the pathogen to a susceptible scion variety, even though the original infected bud failed and rebudding were necessary. Whether or not a somewhat resistant understock could serve to bridge *Verticillium* from an unsuccessful infected bud to a successful re-bud of a susceptible variety remains to be determined. Also warranting study is the role of *Verticillium* in bud failure.

The importance of recognizing the self-evident possibility that systemic fungi or bacteria, as well as viruses, may be bud-transmitted deserves considerable emphasis. This is highlighted by two facts associated with this investigation. First, one of the severe cases studied was initially diagnosed by a plant pathologist, on the basis of symptoms, as a case of either virus streak or virus wilt of roses. Many of the symptoms of virus streak[2] and nearly all those of virus wilt[3] are identical with symptoms of the Verticillium disease observed repeatedly on previously healthy plants inocu-

[1] In an independent study Verticillium symptoms developed within 3 months on branches originating an inch or two below the point of inoculation on 14 of 74 inoculated Better Times plants. *Verticillium* was isolated from each of two such branches on four of nine plants tested.

[2] Brierley, P., and Floyd F. Smith. Mosaic and streak diseases of roses. Jour. Agr. Res. [U.S.] 61: 625–660. 1940.

[3] Grieve, B. J. "Rose wilt" and "dieback." A virus disease of roses occurring in Australia. Austr. Jour. Exp. Biol. and Med. Sci. 8: 107–121. 1931.

lated with pure cultures of *Verticillium*. Second, bud transmission is the sole evidence reported as proof of the virus nature of some rose diseases. The work here reported emphasizes the established principle that bud or graft transmission alone is not acceptable evidence of the virus nature of a plant disease. It may indeed be in order to suggest the critical reexamination of certain reported virus diseases of woody plants for which bud or graft transmission has been offered as proof of their virus nature without adequate examination for possible fungus or bacterial pathogens.

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RECENT DEVELOPMENTS IN STUDIES OF QUICK DECLINE AND RELATED DISEASES[1]

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(Accepted for publication April 5, 1951)

Symptoms of quick decline on susceptible citrus trees in the orchard are very similar to those of trees that have been severely girdled (5). In fact, the work of Schneider (13, 14) has shown that the causative virus in some way brings about a girdling effect near the bud union by blockage and destruction of the food-conducting elements. Trees affected by quick decline, like those girdled experimentally or by such agents as rodents and fungi, usually do not begin to show top symptoms until several months after the initial injury. Because individual trees infected with the virus of quick decline react differently, and the rate of development and degree of severity of symptoms vary with environment, it is difficult at times to determine whether a given tree is or is not infected with quick-decline virus.

The difficulty of correct diagnosis, the long period required for the appearance of symptoms, and the necessity of using budded or grafted trees of susceptible stock-scion combinations in studies of quick decline have delayed progress in the research on this disease. These objectionable features were recognized early in the investigations, and efforts were made to discover better techniques. The obvious need was a consistent, distinctive symptom of the disease that could be induced rapidly, preferably on seedling plants, so as to eliminate the labor and time involved in growing budded or grafted plants; i.e., a rapidly appearing diagnostic symptom on the leaves of some plant that could be grown in large numbers in a relatively short time.

SEARCH FOR LEAF SYMPTOMS OF QUICK DECLINE

In the search for such a leaf symptom, seedlings of many different citrus species and varieties were inoculated by insertion of buds from sweet-orange trees infected with quick decline. This was the technique used by Fawcett and Wallace (6) in demonstrating the virus nature and transmission of quick decline. The test plants were growing in the field and averaged approximately $\frac{1}{4}$ to $\frac{1}{2}$ in. in trunk diameter when inoculated.

The young leaves of some of the inoculated seedlings showed faint, psorosislike veinclearing at times, but it was not established with certainty that such symptoms had been induced by the quick-decline virus. While these studies were in progress, word was received from West Africa that quick decline was present in citrus trees in the Gold Coast Colony, and that lime seedlings developed veinclearing of leaves and other conspicuous symptoms when inoculated from quick-decline-affected citrus trees.[3]

[1] Paper No. 671, University of California Citrus Experiment Station, Riverside, California.

[2] Plant Pathologist in the California Agricultural Experiment Station.

[3] Written communication (January, 1949) from Dr. A. F. Posnette, Head, Pathology Division, West African Cacao Research Institute, to Dr. L. C. Cochran, Senior Pathologist, U. S. Department of Agriculture, Division of Fruit and Vegetable Crops and Diseases.

REACTION OF LIME SEEDLINGS TO QUICK DECLINE

Shortly after this information was received from West Africa large numbers of seedlings of West Indian (Mexican) lime were inoculated with quick decline in southern California, by the same method as that used in the transmission of the psorosis viruses (15, 16). Small pieces of leaf or bark tissue, or small scions from twigs of quick-decline trees, were grafted to the young lime seedlings, and the tops of the seedlings were cut off so as to force new leaf growth from axillary buds. On many plants the first leaves that developed after the top was removed were small, chlorotic, and slightly cupped. As these leaves aged they became somewhat mottled and fre-

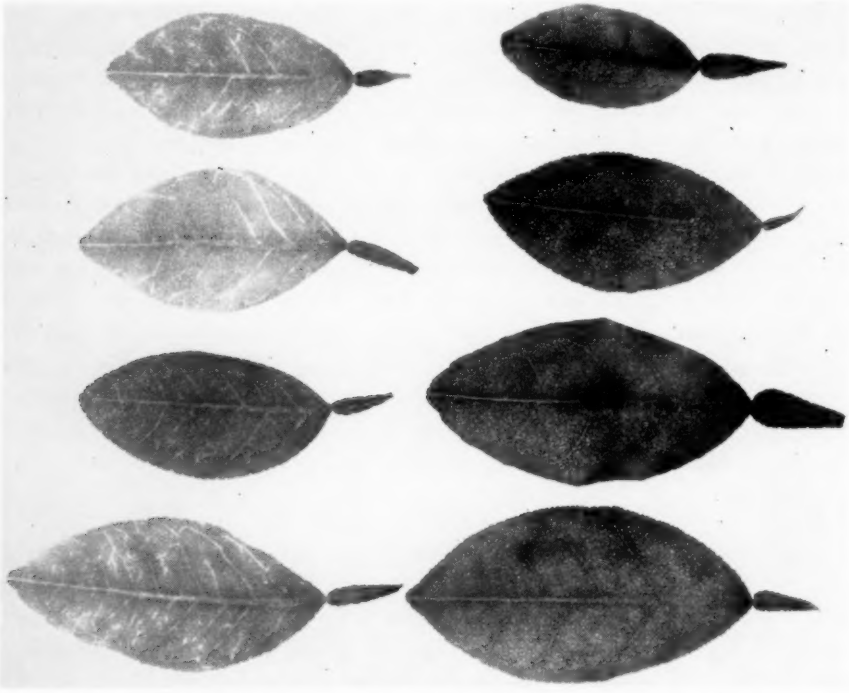

FIG. 1. Left: Veinclearing on young leaves of Mexican lime seedlings inoculated with the virus of quick decline. Right: Symptomless leaves from healthy plants.

quently showed slight veinclearing. Leaves formed later were usually only slightly paler than normal, but many of them manifested a conspicuous veinclearing typical of certain other virus diseases and similar in many respects to the young-leaf symptoms of psorosis on citrus (Fig. 1).

Additional inoculations were made of lime seedlings from several different sources, to eliminate the possibility that the quick-decline trees furnishing inoculum in the first test had been infected, also, with one of the strains of psorosis, or possibly with some other unknown virus. Other lime seedlings were inoculated with the psorosis virus, so that resulting leaf symptoms might be compared with those of quick decline. The symptoms

developing on lime after inoculation from quick-decline trees differed from those induced by the psorosis virus.

The production of these symptoms consistently on lime by inoculation from numerous quick-decline trees seemed to be good evidence that the symptoms observed were induced by the quick-decline virus. But since there was at least a remote chance that the quick-decline trees could be carrying another, unknown virus capable of causing the symptoms shown by the lime seedlings, it still could not be stated with absolute certainty that the symptoms observed had resulted from infection with the virus of quick decline.

Lime seedlings "inoculated" by means of tissue grafts from several symptomless and supposedly virus-free citrus sources outside the quick-decline area failed to reveal the presence of any latent or undetected viruses in any of the sources tested. These limited tests showed at least that no such viruses were generally present in the citrus plantings.

Should the quick-decline virus be present in the diseased limes, inoculations of sweet-orange on sour-orange rootstock from the lime seedlings would, in time, result in typical quick-decline symptoms. Transfers of this type were made from diseased lime seedlings to sweet-orange trees on sour-orange rootstock, and the latter developed quick-decline symptoms typical of that combination.

In these studies it was established that lime seedlings developed certain characteristic symptoms when infected by graft inoculation from known quick-decline trees. It was shown further that the quick-decline virus was established in the diseased limes. Although it is possible that a second plant virus could be present in all quick-decline-affected trees, and that this and not the quick-decline virus caused symptoms on lime seedlings, it has not been possible so far to demonstrate experimentally that such is the case.

THE "LIME DISEASE" IN THE GOLD COAST COLONY AND ITS INSECT VECTOR

In 1949 Hughes and Lister (7) published a brief account of the work on the disease of seedling lime trees in the Gold Coast Colony, referred to above, and reported that in addition to the veinal symptom of leaves and other effects, the wood of the trunks and branches was severely pitted. With this information in mind, the writer examined many of the lime seedlings that developed leaf symptoms after inoculation from quick-decline sources. Externally, the small plants had no abnormal bark condition, but when bark was stripped from the trunks of the diseased plants nearly all of them had numerous small pits or longitudinal depressions in the outer wood (Fig. 2). On some of the seedlings that had been infected about 8 months, the grooves were sufficiently numerous to cause an apparent disorganization of the woody tissues. The inner face of the bark had raised areas that corresponded to the grooves or pits in the wood.

Of particular interest and importance is the disclosure by Hughes and

Lister (7) that the virus of the "lime disease" of the Gold Coast Colony is transmitted by *Aphis tavaresi* (Del Guercio), now known as *A. citricidus* (Kirkaldy), which Meneghini (9) found to be the vector of the tristeza virus in Brazil. This discovery clearly suggested a relation between the lime-disease virus and the tristeza virus.

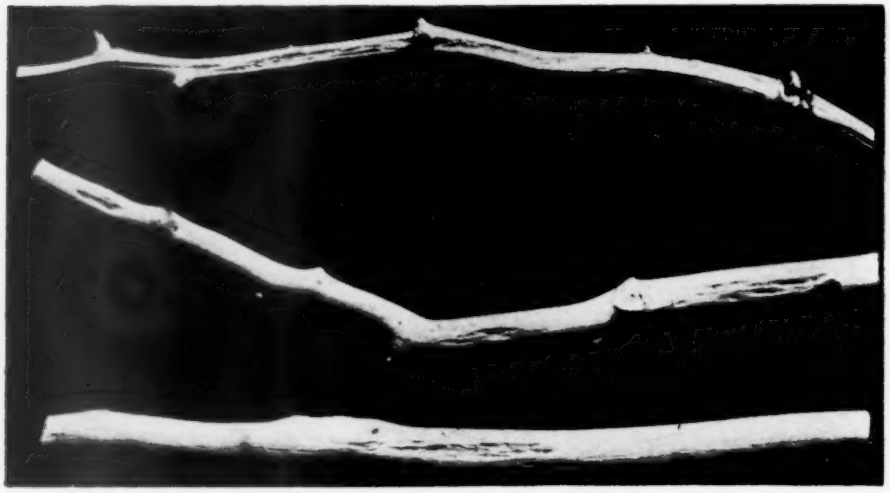

FIG. 2. Main stems of Mexican lime seedlings. Bark removed to show stem-pitting symptoms resulting from infection with the virus of quick decline.

EVIDENCE OF INSECT TRANSMISSION OF QUICK-DECLINE VIRUS TO LIME SEEDLINGS

According to Dickson, Flock, and Johnson (3), *Aphis citricidus* does not occur in the United States. In their search for insect vectors of quick decline, they have made extensive tests with all species of aphids commonly found on citrus in California, as well as with other species for which citrus is not a principal host. One species, *A. gossypii* Glover, the melon aphid, was found by these authors to be a vector of quick-decline virus, but under the conditions of their tests a low percentage of the insect-inoculated plants developed typical quick-decline symptoms (4).

In a preliminary trial approximately 200 melon aphids were caged for 2 days on lime seedlings that had been infected previously by graft-inoculation from quick-decline sources. The surviving insects, about 80 in number, were then transferred to a group of 30 lime seedlings averaging about 2 in. high. Five weeks later the newly formed leaves on some of the lime seedlings were small, chlorotic, and slightly cupped. Seven weeks after inoculation the seedlings were removed from the flat in which they were growing and placed individually in pots. At that time nine of the seedlings had foliage symptoms identical with those on plants inoculated earlier by tissue grafts. These plants continued to show veinclearing, slight chlorosis, and mottling of leaves, and generally remained smaller than healthy plants of the same age. About 6 months from the time of inoculation, small strips of

bark were removed from the diseased lime seedlings; on some of these, symptoms of stem pitting were clearly evident.

In later tests *Aphis gossypii*, transferred directly to healthy lime seedlings without first feeding on quick-decline-affected plants, failed to cause symptoms, as did aphids that fed on psorosis-affected citrus plants before transfer to lime seedlings.

The transmission of the virus responsible for symptoms on lime seedlings by the aphid that has been suspected for considerable time to be the vector of quick-decline virus in California suggests that the symptoms produced on lime by insect and by tissue-graft inoculation are induced by the quick-decline virus. These results also suggest a similarity or relationship between the lime disease in the Gold Coast Colony and quick decline in California, even though different species of *Aphis* serve as vectors in the two localities.

THE STEM-PITTING DISEASE OF GRAPEFRUIT IN SOUTH AFRICA AND ITS RELATION TO THE DISEASE OF LIME

Shortly after publication of the report by Hughes and Lister (7) on "lime disease," McClean (8) published an account of studies on "stem pitting" of grapefruit, a disease which was first described by Oberholzer, Mathews, and Stiemie (11) and which has been of considerable importance for many years in South Africa. McClean demonstrated that the stem-pitting disease of grapefruit is caused by a virus, and that it can be transmitted by tissue grafts and by the insect *Aphis tavaresi* (*citricidus*).

The symptoms on lime seedlings infected with the stem-pitting virus appear to be identical with those described by Hughes and Lister in their study of the lime disease. The fact that the two diseases induce identical symptoms on lime seedlings and have the same insect vector supports the suggestion made by McClean that stem pitting of grapefruit in South Africa and the lime disease in West Africa are caused by the same virus.

It was also suggested by McClean that the disease of sweet lime in Palestine, described as "xyloporosis" by Reichert and Perlberger (12), and a similar disease reported on "Lima da Persia" in Brazil by Moreira (10), may also be caused by the virus that causes the stem-pitting disease of grapefruit in South Africa and the lime disease in West Africa. This suggestion was based on the fact that limes affected with xyloporosis show pitting and grooving of the wood similar to that observed on plants affected by the other diseases. McClean suggested that "If, as seems likely, xyloporosis is the same as stem-pitting [of grapefruit], which is almost certainly the same as the disease of limes, the causal virus of the three diseases should be called the Citrus xyloporosis virus." Recent developments in studies of tristeza in Brazil would apparently place the tristeza disease in the same group. On the basis of relationships already established between tristeza and quick decline, and the data presented in this paper, the quick-decline disease would likewise be included.

RELATIONSHIP BETWEEN THE STEM-PITTING DISEASE AND TRISTEZA

In their studies of tristeza in Brazil, Costa, Grant, and Moreira (1) found that young seedlings of some grapefruits, limes, tangelos, lemons, and sweet orange showed varying degrees of stunting, chlorosis of foliage, and decay of rootlets when infected with the tristeza virus. This demonstrated that it was not necessary to have a bud union present for the virus to have an effect.

A more recent paper by these same authors (2) reports that lime seedlings inoculated with the virus of tristeza by *Aphis citricidus* developed leaf and stem-pitting symptoms which closely resembled the reactions of lime seedlings infected with the virus of the stem-pitting disease in South Africa. They were also able to produce stem-pitting symptoms on grapefruit by inoculation from tristeza-affected trees. Evidence of the relationship between the stem-pitting disease of grapefruit and the tristeza disease appears to be conclusive.

These investigators recognize the possibility that, since tristeza seems to have been introduced into South America from South Africa, the virus of stem-pitting, if distinct from the virus of tristeza, could have been introduced at the same time. However, they have been unable to demonstrate the presence of two distinct viruses in tristeza-affected trees. Because of the marked similarity of symptoms and, particularly, the transmission of the causal virus of both types of disease by the same insect species, these authors conclude (2) that "Unless definite evidence is obtained that a distinct virus is responsible for the stem-pitting disease, it appears more reasonable to consider stem-pitting as a symptom caused by the tristeza virus complex in grapefruit, lime, and some other host plants, especially when these are budded over tristeza-tolerant rootstocks."

DISCUSSION

It has been generally accepted for a number of years that quick decline in California and tristeza in South America are caused by the same virus or by closely related virus strains. Recent independent investigations in South Africa, West Africa, Brazil, and California seem to demonstrate conclusively that the stem-pitting disease of grapefruit in South Africa and the lime disease in West Africa are caused by the same virus that is responsible for tristeza and quick decline. It may well be that the strains of the virus vary in different localities.

The species of aphis that transmits the viruses of the diseases in South Africa, West Africa, and South America is not known to be present in California. *Aphis gossypii*, which at present is the only known vector of the virus of quick decline in California, has probably not been tested sufficiently to determine whether or not it will transmit the viruses that cause the similar diseases. The transmission of the same virus by different species of aphids is not uncommon and, at present at least, cannot be accepted as evidence that the quick-decline virus is distinct from that causing the similar diseases.

It still remains to be demonstrated that xyloporosis is a transmissible disease. Results of investigations of xyloporosis in Palestine and Brazil, and observations of a similar condition on various citrus species in California, suggest to the writer that xyloporosis is a heritable condition that is probably accentuated by certain environmental factors, particularly stock-scion relationships. Additional study is needed to establish the cause of xyloporosis.

Certain characteristics of xyloporosis suggest that even if it is a virus disease the causal virus is not the same as that responsible for the other diseases discussed in this paper. It has been reported from Palestine (12) that xyloporosis symptoms occur commonly on seedlings of sweet lime or on trees of Shamouti orange, sour lemon, grapefruit, and mandarins on sweet-lime rootstocks. If this condition is caused by the quick-decline (tristeza) virus, it is difficult to explain the absence, in Palestine, of the typical symptoms on citrus combinations of scion and rootstock which are seriously affected in California and South America. Furthermore, it is stated (12) that trees of sweet orange on sweet-lime rootstock affected with xyloporosis recover when inarched to sour orange. Moreira (10) also reported that xyloporosis-affected trees of sweet orange on "Lima da Persia" root recover when inarched to sour orange or when topworked to sweet orange. These results would not be anticipated if xyloporosis were the same as quick decline or tristeza.

Until further information is available on the transmission and insect-vector relationships of xyloporosis, it cannot be concluded that this condition is closely related to these other diseases, namely, quick decline, tristeza, stem pitting of grapefruit, and lime disease.

SUMMARY AND CONCLUSIONS

West Indian (Mexican) lime seedlings develop diagnostic foliage symptoms within 4 to 6 weeks after inoculation with the quick-decline virus. This provides a short-time test that finds many uses in studies of this disease. The symptoms appear consistently and distinctly under environmental conditions that have not been conducive to symptom expression of budded sweet orange on susceptible rootstocks. The use of lime seedlings as an indicator plant will be of particular value in insect-vector studies.

The symptoms induced on lime seedlings by the quick-decline virus appear to be identical with those described for the "lime disease" in the Gold Coast Colony and for the stem-pitting disease in South Africa, and they closely resemble the effects induced by tristeza in Brazil.

From a study of the published accounts of investigations of the disease known as "xyloporosis" in Palestine and Brazil, the writer suggests that there is not sufficient evidence to conclude that this disease is related to the other diseases discussed.

An analysis of data from independent studies in South Africa, West Africa, Brazil, and California leads to the suggestion that the respective

stem-pitting disease, disease of limes, tristeza, and quick decline are caused by the same virus or by closely related strains of the same virus.

Early observations on quick decline in California, tristeza in South America, and related disorders in other citrus-producing areas indicated that this malady affected only budded trees of certain combinations of scion and rootstock. The research results of various investigators, which have been summarized in this paper, demonstrate that the same virus can also induce specific disease symptoms on seedlings of certain citrus species and on certain budded combinations that have not shown the typical symptoms of quick decline or tristeza. On the basis of symptoms alone, descriptions of at least three apparently different diseases have appeared in the literature. It now seems well established that what were thought to be three distinct diseases are merely variations in reaction of different hosts or host combinations (scion and stock) to infection with the same virus.

In view of these developments, the previously established ideas of control or prevention must be revised. On the basis of earlier observations it was believed that injury could be avoided by planting scion-stock combinations of citrus that were not susceptible to the breakdown of the phloem or food-conducting tissues in the vicinity of the bud union. In South Africa the decline of grapefruit which accompanies the stem-pitting symptom occurs on grapefruit seedlings as well as on grapefruit budded on Rough lemon rootstock. Probably some grapefruit varieties will be found subject to this particular type of injury regardless of which rootstock is used, even though they may not show the type of symptoms usually associated with quick decline or tristeza.

The threatened destruction of the lime industry in the Gold Coast Colony demonstrates the serious potentialities of invasion by the quick-decline virus into citrus-producing areas which formerly would have been considered relatively safe.

The developments summarized in this paper clearly emphasize that the preventive control of this type of disease is much more complicated than it was formerly believed to be. It is fortunate, however, that this information has become available at a time when it can be made use of in the extensive varietal and rootstock tests now being made in both Brazil and the United States.

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THE USE OF RADIOISOTOPES IN DETERMINING THE DISTRIBUTION OF BACTERIUM STEWARTII ERW. SMITH WITHIN CORN PLANTS[1]

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(Accepted for publication April 16, 1951)

With recently increased production and more widespread distribution of radioactive isotopes, a new research tool has been introduced into the biological sciences. Especially adaptable to techniques involving the use of radioactive materials is the problem of the distribution of pathogenic microorganisms or their toxic products within the tissues of the host. Studies of this type have been made possible by the development of radioactive or "tracer" elements which can be incorporated into media suitable for the growth of bacteria and fungi.

Prior to the development of these materials and the techniques of their use, it was necessary, in studies of bacterial distribution within plants, to attempt to isolate the pathogen from the host. With each attempted isolation that particular plant was partially if not totally destroyed. Though unavoidable, this has certainly not been wholly satisfactory, especially in studies of the rates of distribution, because differences, either environmental or genetical, might occur even among individuals of relatively pure strains. Even when total destruction of the host could be avoided, relatively slight damage might cause physiological changes which would alter the distribution rate.

Before radioisotopes were used in studying the actual distribution of bacteria within the host, it was considered necessary to determine 1) what effects various concentrations of radioactivity had on the growth of the particular isolate of *Bacterium stewartii* Erw. Smith being used, and 2) whether suitable concentrations of radioactive elements would accumulate within the bacterial cells.

REVIEW OF PUBLICATIONS

A number of studies have been made of mutations in *B. stewartii* following irradiation. Lincoln and Gowen (7) have demonstrated that the types of variants found after X-ray irradiation are similar to the types found in nonirradiated cultures, but that the rate of variation is 600 times greater in irradiated cultures than in nonirradiated.

In addition to the above-mentioned studies on effects of radiation on the growth rate and form of *B. stewartii*, studies have been made of these effects on other bacteria. The literature on X-ray studies is very extensive and reviews have been published by Pugsley, Oddie, and Eddy (11), by Lea,

[1] Excerpt from a thesis presented to the Department of Botany and Plant Pathology, The Ohio State University, in partial fulfillment of the requirements for the degree, Doctor of Philosophy.

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Haines, and Bretscher (5), and by Lorenz and Henshaw (8). Most of the early workers, including Minck (9) and Wittlin (17), believed that X-rays had little effect on bacteria. Rieder (12), soon after the studies of Minck and Wittlin, exposed a number of different bacteria to X-rays and observed a definite killing effect after irradiation.

In the area of study concerning the effects of emanations from radioactive elements, the first experiments were by Pacinotti and Porcelli (see Spencer, 13), who exposed several different organisms to preparations of uranium and reported that the organisms were killed after 24-hr. exposure. Strebel (15) exposed *Serratia marcescens* Bizio on agar to rays from radium, and after different periods of irradiation the degree of growth was compared with that of nonirradiated cultures. In general, the growth of cultures exposed to such radiation was inhibited. These observations with radium and its salts were confirmed and extended by the work of Pfeiffer and Friedberger (10).

Among more recent investigations are those of Spencer (13), who has reported that beta-rays and gamma-rays retarded the growth of *Salmonella typhosa* (Zopf.) White, *Proteus vulgaris* Hauser *X 19*, and *Streptococcus pyogenes* Rosenbach for a short time but that in 24 hr. the growth was equal to that in the control tubes. Lea, Haines, and Coulson (6) found that bacteria exposed to alpha- or beta-particles died in an exponential manner which was independent of the temperature but proportional to the intensity of the radiation. These workers found that, contrary to results of some of the earlier investigators, gamma rays are lethal to bacteria in aqueous suspensions and death occurred exponentially but at a much slower rate.

Most of the publications on the general subject of Stewart's disease contain discussions of the distribution of the causal organism, *B. stewartii*, within the host plants. Such investigators as Stewart (14), Elliott (3, 4), Dickson (2), and Chester (1) observed that early in the development of the disease the vascular elements of the plant are invaded by the pathogen. After the invasion of the xylem vessels the ground parenchyma surrounding the vascular bundles may be invaded, while the bacteria continue to grow in the vessels and keep pace with the growth of the host. The distribution within the host is general, with little or comparatively little localization of the bacteria, although Wellhausen (16) has reported, in the moderately susceptible Golden Bantam and white flint corn lines, a definite radial arrangement of heavily lignified cells around the point of invasion which he considers to be a "defense mechanism."

MATERIALS AND PROCEDURES

The tolerance of *B. stewartii* to radioactivity concentrations was determined by a number of separate experiments. In the first of two representative tests, the media used for the cultivation of the bacteria were potato-dextrose broth and Difco brain-heart infusion.

These media were tubed in 10-ml. quantities and to some of the tubes

0.25 ml. of radioactive phosphorus was added in the form of $Na_2HP^{32}O_4$, having a radioactivity of 160 microcuries per ml. This gave a medium with an initial radioactivity of 4.00 microcuries per ml. The media in the tubes were inoculated with one loopful of a 48-hr.-old broth culture of *B. stewartii*. After incubation for 48 hr., the radioactivity had decreased by approximately 7 per cent, or to 3.72 microcuries per ml. The amount of bacterial growth was measured visually in comparison with control tubes which contained no radioactive phosphorus.

In the second experiment MacLeod's medium[3] was used. The P^{32}, as in the previous experiment, was in the form of $Na_2HP^{32}O_4$ having a radioactivity of 37.5 microcuries per ml. The radioactive solution was so diluted that the concentration of nutrients in the media was kept nearly constant, while the initial range of radioactivity varied from 0 to 7.4 microcuries per ml. For each level of radioactivity concentration two tubes of medium were inoculated with one loopful each of a 24-hr.-old culture of *B. stewartii* growing in beef extract-peptone broth. After incubation at room temperature (22°–25° C.) for 24 hr., duplicate dilution series were prepared from each tube, in sterile water, and samples of each were placed in sterile Petri dishes. Melted, cooled beef extract-peptone agar was then poured into the plates. These were incubated for 3 days at room temperature, and the number of colonies developing were counted. From these data the effect of radioactivity on the growth rate of the bacteria was computed.

The cultures used were transplants from Culture Number 8199 of the American Type Culture Collection.

It was decided to use radioactivity concentrations of 0.37–1.00 microcuries per ml. in the plant inoculation experiments since it seemed that this amount of radiation did not decrease the growth rate of *B. stewartii* sufficiently to materially affect the development of disease following inoculation of the corn plants.

To determine the rate of distribution of the bacteria within the tissues of the host, two corn inbreds were used, 651 (M 8 1947), a susceptible variety, and Purdue 39A, a resistant variety.[4] The inoculated plants were of two ages and of various heights, measured in millimeters from the soil surface to the highest leaf tip. The inoculated plants were divided into two sets, one of which was placed in a moist chamber in a dark room. For the moist chamber, a large wire laundry hamper was inverted and covered with several layers of cheesecloth; the cloth was kept saturated with water flowing through tubes which opened near the edge of the base of the hamper. A Geiger monitor tube was introduced through openings cut in the cheese-

[3] The medium was prepared as follows: Sodium chloride, 5 gm.; ammonium sulfate, 4.72 gm.; dipotassium phosphate, 2.72 gm.; dextrose, 2 gm.; l-asparagine, 2 gm.; water, 1,000 ml. To this there was added 1 ml. of a solution containing 1 gm. ferric chloride, 1 gm. magnesium chloride, and 1 gm. calcium chloride per l. of water. The pH of this medium after autoclaving was 7.4 as determined by a Beckman glass electrode pH meter.

[4] The seeds for these plants were generously supplied by Dr. Glenn M. Smith, Associate Geneticist, Department of Botany and Plant Pathology, Agricultural Experiment Station, Purdue University, Lafayette, Indiana.

cloth. Different numbers and sizes of stakes were used in the pots so that they could be differentiated by touch.

After growth for 48 hr. in MacLeod's medium containing not less than 0.37 nor more than 1.00 microcuries of radioactivity per ml., the cultures were transferred aseptically to sterile centrifuge tubes. The supernate was poured off, and the cells were resuspended in sterile medium and recentrifuged three times. After this amount of washing the supernate contained sufficient radiation to cause Geiger monitor responses slightly but not significantly greater than were caused by the background alone. The amount of radiation was checked in a Model 64 Scaler, manufactured by Tracerlab, Inc.

Centrifugation was begun with 45 ml. of 48-hr.-old cultures, and the packed, washed cells were resuspended in MacLeod's medium to a volume of 5 ml.

For plant inoculation with this concentrated cell suspension, small glass tubes were prepared from $\frac{1}{8}$-in., thin-walled glass tubing heated and stretched so that the ends were drawn abruptly into capillaries; these were inserted into the plant stems near the surface of the soil and filled with 1/10-ml. quantities of the suspension of radioactive cells.

The movement of the bacteria within host tissues was followed with a Model SU 3A Laboratory Monitor. When isolations were made to recover the bacteria from the host, tissues were removed from the plant, surface-sterilized in a calcium hypochlorite solution, dropped into tubes of Difco brain-heart infusion broth, and incubated for 24 hr. Streak dilution plates were then made to beef extract-peptone agar plates.

Radioautographs were prepared to determine the corn plant tissues involved in the transport of the bacteria. Inoculated plants were cut at the surface of the soil and then, depending upon the size, the whole plant or parts of it were placed on DuPont Type 508 X-ray film, covered with smooth metal plates, and weighted. After a number of hours, which varied with the radiation intensity, the plant parts were removed and the film was developed with the usual X-ray developers.

RESULTS

In the experiment designed to determine the growth responses of *B. stewartii* to radioactivity, in which potato-dextrose broth and brain heart infusion media were used, there was no apparent reduction in the rate or amount of growth. Growth was measured by visual comparison of control tubes with tubes of media containing 4.00 microcuries of radioactivity per ml. of medium.

The results of the experiment in which P^{32} was incorporated in MacLeod's medium are in table 1. The intensity of radiation was varied, and the rate of increase of the bacteria was determined by plate counts.

Following tests to determine the radioactivity tolerance of *B. stewartii*, plants were inoculated by the previously described methods and the rate of

TABLE 1. *Effect of various concentrations of radioactive phosphorus on the growth rate of Bacterium stewartii. All of the values given represent the average for two plates. Colonies were counted on only those plates with 200 to 400 colonies. MacLeod's medium.*

Tube	Radioactivity concentration (mc/ml.)	Number of bacteria per ml. of culture
A–18	7.4	10,600,000
A–8	7.4	17,000,000
A–7	3.7	30,000,000
A–13	3.7	25,900,000
A–11	0.74	34,900,000
A–1	0.74	34,400,000
A–15	0.37	56,300,000
A–3	0.37	69,000,000
A–9	0.00	52,000,000
A–19	0.00	47,000,000

TABLE 2. *Rate of movement of Bacterium stewartii within its host as determined by the use of bacteria made radioactive with* P^{32}

Illumination and Variety	Plant	Size in mm.	Time interval, in minutes, between inoculation and the detection of 100 disintegrations per min. at leaf tip	Result of reisolation attempt
Light				
651	1–A	485	95	Positive
	2–A	320	65	Positive
	3–A	410	80	Positive
	4–A	490	110	Positive
39–A	5–A	435	70	Positive
	6–A	250	45	Positive
	7–A	320	65	Positive
	8–A	460	100	Positive
651	1–B	540	215	Positive
	2–B	425	120	Negative
	3–B	495	95	Negative
	4–B	525	190	Positive
39–A	5–B	480	300+	Negative
	6–B	340	125	Positive
	7–B	310	145	Positive
	8–B	435	95	Positive
Dark				
651	1–A	285	630	Positive
	2–A	320	1050	Positive
	3–A	490	840	Positive
	4–A	415	930	Negative
39–A	5–A	255	930	Positive
	6–A	340	870	Positive
	7–A	345	870	Positive
	8–A	470	780	Positive
651	1–B	635	1440	Negative
	2–B	505	1440	Negative
	3–B	425	1440	Negative
	4–B	420	1440	Negative
39–A	5–B	445	1440	Negative
	6–B	460	1440	Negative
	7–B	385	1440	Negative
	8–B	465	1440	Negative

movement of the bacteria through the host tissues was determined (Table 2). Figure 1 is a radioautograph of leaves from one of the plants.

DISCUSSION

The results of these experiments made it apparent that if radioisotopes have sufficient radioactivity they exert a definite depressing effect on the growth rate of *B. stewartii* as measured in terms of living bacteria. There was, however, a wide range of radioactivity concentrations which did not have a marked effect on reproduction of *B. stewartii*. The upper limit of this concentration was more than adequate to permit the incorporation into bacteriological media of sufficiently radioactive elements to cause bacteria growing in the media to become measurably radioactive. The depressing effect of the isotopes was not manifest at the same level of concentrations in

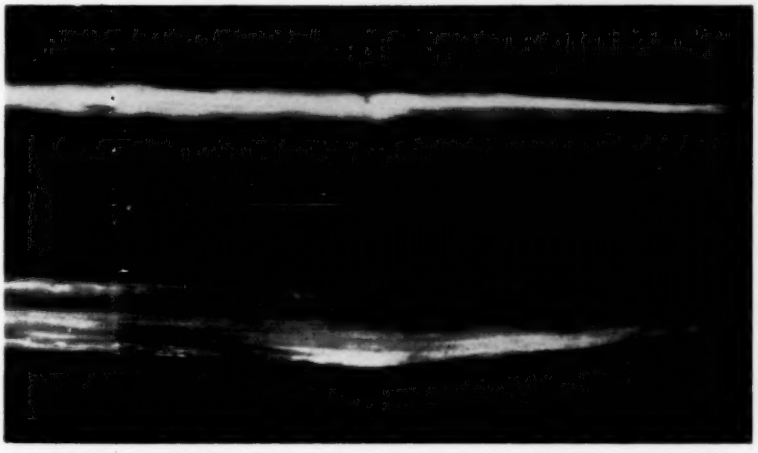

FIG. 1. Radioautograph of leaves taken from a corn plant inoculated with a radioactive culture of *B. stewartii*.

organic as in inorganic media. Bacteria growing in media rich in organic matter were apparently not numerically affected by concentrations of radioactivity that had a considerable effect when the bacteria grew in inorganic media.

Following this preliminary work, the dissemination of *B. stewartii* within the tissues of its host was studied. The data obtained indicate that even in resistant inbreds the bacteria are widely distributed in the plants, and resistance does not depend upon the localization of the infection. There was no significant difference between the rate of movement and the extent of distribution of the bacteria within the hosts, in susceptible and in resistant varieties of corn. This, of course, refers only to the initial distribution immediately following inoculation. The rate of movement suggested transport through the xylem vessels of the plant; therefore, the rate of transpiration was artificially decreased when water was withheld from the soil and plants were grown in the dark in a moist chamber. The transport of bacteria by the transpiration stream was confirmed.

The technique used in these experiments is a newly developed one whose ultimate usefulness is undoubtedly great, especially if it is found that bacteria and fungi will incorporate selected isotopes into the toxins which they produce.

SUMMARY

The growth or reproduction of *B. stewartii* is not seriously limited by radioactivity concentrations which permit assimilation of easily detectable amounts of radioactive phosphorus.

The rapidity of the distribution of *B. stewartii* within inoculated corn plants is correlated with the rate of transpiration of the corn plant.

As shown by the use of radioactive bacteria, there is no appreciable difference in the rate or extent of distribution of *B. stewartii* within susceptible and resistant inbreds.

The feasibility and practicality of using radioactive tracer elements in phytopathologic studies is demonstrated.

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EFFECT OF PASTEURIZATION ON BLACK ROT OF SWEET POTATOES

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(Accepted for publication April 27, 1951)

In the earlier experiments on heat treatment (pasteurization) for control of black rot of sweet potato, caused by *Endoconidiophora fimbriata,* the effect of treatment for 1, 2, and 3 days was determined.[2] Holding non-cured sweet potatoes at 110° F. for 1 day was lethal to the black-rot pathogen and apparently did not affect the quality of the sweet potato. This paper records 1) results of further study of the effect of shorter treatments and lower temperatures on black rot; and 2) the effect of the treatments on the quality of sweet potatoes after cooking and their production of sprouts.

Sweet potatoes of the Maryland Golden variety, grown near Salisbury, Maryland, were taken to the laboratory as soon as dug and held at room temperature (approximately 75° F.) until the next day. They were then carefully examined for black-rot lesions, and all showing any infection, about 2 per cent, were discarded. Three half-bushel baskets of potatoes were used for each treatment. Temperatures were automatically controlled and usually varied less than 1 degree.

Weight losses and losses from rots, on a weight basis, of sweet potatoes in this experiment are in table 1. Percentages are based on original weight before treatment. When black rot was present it was recorded as such and other rots on those same roots were not recorded. This system has the advantage that one root is counted only once, but probably less of "other rots" is recorded than actually occurred.

Treatment at 110° F. for 16 and 24 hr. (Table 1) prevented development of black rot but treatment at 110° for 8 hr. had little or no effect on black-rot incidence. In the earlier report[2] it was noted that even though the rot had already started, its development ceased after heat treatment at 110° for 24 hr. At 105°, the 24-hr. treatment was effective in preventing black rot, whereas treatment for 16 hr. reduced the disease but did not entirely prevent it (Table 1). Treatment for 8 hr. at 105° and for 8, 16, or 24 hr. at 100° did not control the disease.

Black rot developed freely in lots not pasteurized but cured 10 days at 85° F. and 85 per cent relative humidity. It did not develop in the lot not pasteurized but cured at 95° F. and 85 per cent r. h. for 10 days. It may be that conditions were unfavorable for the development of lesions during curing at 95° and storage at 55° even though some infection had occurred.

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[2] Kushman, L. J., and J. S. Cooley. Effect of heat on black rot and keeping quality of sweet potatoes. Jour. Agr. Res. [U.S.] 78: 183–190. 1949.

Kawarada and his associates,[3] reported that curing at 95° F. controlled black rot.

The rots other than black rot (Table 1) were present on both treated and nontreated potatoes. The record of the rots and of the general condition of all the treated roots was made after more than 3 months' storage.

Since the sweet potato is extremely sensitive to any unfavorable storage environment, undoubtedly the roots were undergoing many changes during this time that would affect susceptibility to decay.

Because weight loss is greatly influenced by the kind and amount of decay, weight losses during pasteurization, curing, and storage were recorded separately. The amount of decay as shown in table 1 tends to obscure the

TABLE 1.—*Weight loss and loss from rots in Maryland Golden sweet potatoes after pasteurization, curing for 10 days at 85° F., and storage for 90 days at 55° F.*

Pasteurization[a]		Percentage weight loss				Percentage rot		
Temperature (° F.)	Period (hours)	After pasteurization	After curing	After storage	Total	Black rot	Other rots	Total
100	8	1.1	11.1	4.6	16.8	12.7	22.3	35.0
	16	1.3	6.8	4.1	12.2	15.9	19.2	35.1
	24	2.9	7.4	3.9	14.2	5.9	19.5	25.4
105	8	1.1	9.0	4.7	14.8	12.5	20.0	32.5
	16	1.8	8.0	4.4	14.2	3.3	21.5	24.8
	24	2.7	8.1	5.4	16.2	0.0	20.9	20.9
110	8	1.1	12.7	5.4	19.2	15.2	17.3	32.5
	16	1.8	9.9	5.5	17.2	0.0	25.2	25.2
	24	1.4	11.9	7.1	20.4	0.0	31.6	31.6
None (cured at 85°)		.....	7.2	5.9	13.1	22.7	22.4	45.1
None (cured at 95°)		.....	7.4	9.4	16.8	0.0	28.1	28.1

[a] Pasteurization treatments were given Oct. 21, 1948, and were followed by curing for 10 days at 85° F. and 85 per cent r. h. before storage at 55° F. until January 19, 1949.

relation between the pasteurization treatment and the weight loss. In evaluating the influence of the heat treatment on weight loss it is necessary, therefore, to consider the amount and nature of the rots. The total weight loss was somewhat greater in some lots given the pasteurization treatment than in the unpasteurized lot, but this loss was slight in comparison with possible black-rot losses.

When a representative sample from each treatment was cut lengthwise, sweet potatoes given pasteurization treatment had as good internal appearance as the checks and there was no discoloration of flesh of those given any of the treatments.

A sample from each lot was baked and judged for eating quality by persons of discrimination. All the lots, regardless of treatment, were consid-

[3] Kawarada, M., S. Matsubora, and S. Sakurai. Construction and equipment of sweet-potato storage house and storage of sweet-potato by electricity with special reference to the prevention of black rot disease. (Title in English.) Jour. Hort. Assoc. Japan. 17 (1–2): 69–72. 1948.

ered acceptable as to taste, but some of the judges considered the potatoes treated at 110° F. less desirable.

Sprout yield from heat-treated sweet potatoes is a good index of their vitality, and is important from the standpoint of propagation. Three potatoes, as uniform as possible in size and shape, from each of the three replications in experiment 2 were bedded in moist sand and held at 85° F. for about 3 weeks; some of the sprouts were then about planting size. The number of sprouts was not appreciably affected by the different heat treatments and even the most severe treatment did not kill the sweet potatoes.

DISCUSSION

The results reported indicate that it is possible to kill the black-rot pathogen in sweet potatoes by a treatment that does not lessen their value for seed purposes or impair their eating quality. Few instances are on record in which a pathogen has been killed in the host without destruction of the host.

The fact that black rot did not develop in storage with 10 days' curing at 95° F. may explain a recent observation in a commercial storage house having a high ceiling. The room was known to have become overheated early in the curing period but the exact temperature was not known. When the room was emptied the next spring, no black rot was present on sweet potatoes near the ceiling, but it was abundant in those near the floor. Possibly the temperature near the ceiling was high enough to kill the black-rot pathogen but that near the floor was not.

Black-rot is especially serious on early shipments of noncured sweet potatoes. When even a very little black rot is present in the crop at digging time it usually causes severe losses during transportation and marketing. Thus, a pasteurization treatment, usable on a commercial scale immediately after potatoes are dug, might prevent tremendous losses from spoilage by black rot during shipping and might save considerable labor in sorting.

SUMMARY

Sweet potatoes from crops having some black rot at harvest were given heat treatment (here called pasteurization) for 8, 16, and 24 hr. at each of three temperatures, 100°, 105°, and 110° F. No black rot developed on those held at 110° and 105° for 24 hr. and at 110° for 16 hr. In the lot held at 105° for 16 hr. black rot was not completely controlled, but there was considerably less than in the unpasteurized lot. The other treatments did not materially lessen the amount of black rot. Heat treatment did not prevent the development of rots other than black rot and did not perceptibly impair eating quality or inhibit production of sprouts.

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WILT-INDUCING POLYSACCHARIDES DERIVED FROM CROWN-GALL, BEAN-BLIGHT, AND SOFT-ROT BACTERIA

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(Accepted for publication May 1, 1951)

In the past, some attention has been paid to the toxin theory of wilting, in which the wilt-inducing ability of various bacteria and fungi was ascribed to toxins secreted by these wilt-inducing organisms. Recent work has stressed the separation of various chemical compounds from culture filtrates of wilt-inducing organisms. The wilt-inducing capacities of these compounds have been demonstrated, and the compounds have been shown to be the active principles of the wilt-inducing culture filtrates.

The literature on the wilt problem has been reviewed by Brown (3), Gottlieb (6), and Harris (7). Investigations have recently been made by Thomas (13), Clauson-Kaas et al. (4), Dimond (5), and Hodgson, Riker, and Peterson (8, 9). Further work by the last-mentioned scientists (10) has indicated that these wilt-inducing compounds need not be derived from specific microorganisms. They have shown that artificially synthesized polymers, such as Carbowax, are active wilt-inducing agents whose ability to cause wilting is apparently dependent upon their molecular size.

The present paper describes a number of wilt-inducing polysaccharides derived from non-wilt-inducing phytopathogenic bacteria, and gives further evidence of the lack of correlation between the source of a compound and its ability to cause wilting.

PREPARATION OF THE ENDOTOXINS AND POLYSACCHARIDES

Boivin et al. (1, 2) and Mesrobeanu (11) reported that a polysaccharide-polypeptide-lipid complex derived from *Agrobacterium tumefaciens*, the crown-gall bacterium, was capable of inducing typical tumors when inoculated into wounded sunflower plants, and that the endotoxin complex could be cleaved by mild acid hydrolysis into polysaccharide and polypeptide-lipid components.

During studies of the biological and chemical properties of the uncleaved endotoxin, it was decided to study also the chemical and biological properties of the acid-cleaved material. Since it was known that previously isolated wilt-inducing substances were often polysaccharides, the wilt-inducing properties of the cleaved and uncleaved endotoxin were given special attention.

Four strains of *A. tumefaciens* were mixed and grown on a Riker and Riker mineral yeast medium (12) modified by the addition of gentian violet 1:500,000, and a defoaming agent, "Fomex," 1:6,000. Each 10-liter Pyrex bottle was charged with 6 l. of the medium, plugged, autoclaved for 1 hr., and cooled overnight at 0° C. Each bottle of cooled, sterile broth was seeded with 10 ml. of a heavy bacterial suspension derived from 24-hr. slants

of the four virulent strains of the pathogen. The bottles were incubated for 48 hr. at 27° C. in a large constant-temperature bath, and were constantly aerated during the incubation period by sterile washed air bubbling through them.

After 48 hr. the broth was combined and the bacterial mass separated by centrifugation in a Sharples centrifuge at 30,000 r.p.m. The bacterial mass was thoroughly washed in sterile distilled water, lyophilized to dryness, and stored at – 18° C. Yields were 15–20 gm. dry weight of virulent bacteria per 24 l. of broth.

Complete endotoxin was prepared by the Boivin trichloracetic acid technique (2) as follows: 10- to 20-gm. lots of dried bacterial cells were mixed in chilled 4 per cent trichloracetic acid, 2 ml. of acid being added for every 0.2 gm. of bacterial cells. The mixture was shaken vigorously and placed at 0° C. for 3 hr., with occasional shaking. The bacterial cells were then spun down in a Sorvall centrifuge at 13,000 r.p.m. The supernatant containing the endotoxin dispersed in trichloracetic acid was dialyzed against running tap water for 48 hr., acidified with 0.1 N HCl to a methyl red end point, and dialyzed against distilled water at 0° C. for 24 hr. The dialysate was then lyophilized to dryness and stored as a pale white to purple fluffy powder. Yields averaged about 3 per cent of the initial dry weight of bacteria.

The complete endotoxin thus obtained was a polysaccharide-polypeptide-lipid complex which was nondialyzable, soluble in organic solvents, insoluble in water, highly opalescent, and pale yellow in color. The material gave a positive Biuret test, a negative Ninhydrin test, and a positive Molisch test. The polypeptide-lipid fraction was rapidly dialyzable, was soluble in organic solvents but insoluble in water, and gave a positive Sakaguchi test (Arginine), positive Biuret and Ninhydrin tests, and a negative Molisch test. The polysaccharide fraction was slowly dialyzable, insoluble in organic solvents but soluble in water, and had an optical rotation of plus 55 degrees. It gave a positive Molisch test and negative Fehling's, Ninhydrin, and Biuret tests. The chemical composition of the complete endotoxin expressed on a percentage basis was as follows: fat, 18–20; sugar, 40–45; ash, 3.0; P, 1.0; N, 1.8; H, 7.0–8.0; C, 44–45. These chemical and physical characteristics were identical with those reported by Mesrobeanu (11) for the endotoxin of *A. tumefaciens*.

The complete endotoxin of *A. tumefaciens* was split into its polysaccharide and polypeptide-lipid components by acetic-acid hydrolysis as described by Mesrobeanu (11). Complete endotoxin (100 ml.) was acidulated by the addition of 1 ml. of 5 N acetic acid, refluxed on a steam bath until a white precipitate formed, and then allowed to cool. The solid lipoidal material (white precipitate) settled out, leaving a clear, nonopalescent, polysaccharide-containing supernatant. The polysaccharide was recovered by concentrating the supernatant to one-tenth its volume on a steam bath, then adding the concentrate, drop by drop, with constant stirring, to 4–5 volumes

of chilled acetone. After several reprecipitations, the polysaccharide was washed in alcohol and ether and was dried at 50° C. to a pure white powder. The powder was completely soluble in water; it gave a positive Molisch test, and a negative test with iodine and Fehling's solution. It was slowly dialyzable through a cellophane membrane, and had an optical rotation of plus 55 degrees. The polysaccharide was hydrolyzed by refluxing for 1 hr. with 1 N HCl and was found to consist of 98 per cent glucose units and a small amount of other residues, probably galacturonic acid.

The endotoxins of two other non-wilt-inducing phytopathogenic bacteria, *Erwinia carotovora* and *Xanthomonas phaseoli*, were similarly prepared. Polysaccharides derived from these endotoxins were similar in nature to the one derived from *Agrobacterium tumefaciens*.

WILT-INDUCTION BY THE ENDOTOXINS AND POLYSACCHARIDES

Cuttings of sunflower (Russian Mammoth) and tomato (Bonny Best) were used to test the wilt-inducing capacity of the complete endotoxins and the derived polysaccharides of *Agrobacterium tumefaciens*, *Xanthomonas phaseoli*, and *Erwinia carotovora*. Cuttings from 21-day-old tomato plants with 2 to 3 leaves and from sunflower plants with the second true leaf were placed in tap water for an hour before each trial. Aqueous solutions of the complete endotoxins and their polysaccharide derivatives were made up in five concentrations ranging from 1:1,000 to 1:20,000, and buffered at pH 6.2 with a Sorensen phosphate NaOH buffer. Twenty ml. of each solution were placed in a vial, and one cutting was placed in each vial after its base was recut under tap water. Five cuttings of each plant species were used for each concentration and each test was repeated at least four times. All tests were held for 48 hr. in an insulated room at a temperature between 58° and 62° F. and lighted with a 500-watt bulb. The air was circulated by a small fan.

The results, recorded after 10 and 48 hr., show that the polysaccharides derived from the respective endotoxins of these pathogens were capable of inducing wilting within 48 hr. in sunflower and tomato-stem cuttings at all concentrations used. After 10 hr. at polysaccharide concentrations of 1:1,000 to 1:10,000, the entire leaf of the sunflower cuttings was involved but only the leaflets of the tomato cuttings were affected. Immersion in these concentrations for 48 hr. caused wilting of the entire leaf and stem in both types of cuttings. However, at a concentration of 1:20,000 no tomato stems wilted after 10 hr. and only the leaflets did so after 48 hr.

Wilting was progressive: the leaflets or leaf blades first, then the petioles, and finally the stems. Wilting was also reversible: cuttings recovered turgidity when removed from the test solutions to distilled water, their bases being recut. However, none recovered when the bases were not recut at least $\frac{1}{2}$ in. above the initial cut surface. Although all cuttings recovered their turgidity, the leaves of sunflower cuttings often remained in an epinastic position. This evidence seemed to indicate that the polysaccharides

caused wilting through a mechanical block, recovery taking place when that block was removed.

In a test to demonstrate that the polysaccharide was the only active wilt-inducing component of the endotoxin, the wilt-inducing capacity of the polypeptide-lipid portion was negative. The wilt-inducing capacity of the polysaccharide was slowly lost upon dialysis against distilled water. Heating of the endotoxin and polysaccharide did not destroy the wilt-inducing principle, but hydrolysis with 1 N HCl for 2 hr. caused complete loss of the active principle. These facts indicated that the polysaccharide was the active wilt-inducing principle, and that the action of this principle depended largely upon its molecular size.

DISCUSSION

Wilting of cuttings may be induced by the action of the endotoxins and derived polysaccharides obtained from three species of phytopathogenic bacteria. The polysaccharide component of the endotoxin is apparently the active wilt-inducing principle. Undoubtedly, the polysaccharide obtained from the endotoxin of the crown-gall organism is not the same as that obtained by Hodgson et al. (9) from the filtrate of growing cultures of the crown-gall bacterium. The polysaccharide described by these workers was secreted into the medium, had a low negative optical rotation, was made up entirely of glucose units, and had a yield 600 times that of the polysaccharide described in this paper.

Thus, two different polysaccharides obtained from the same microorganism have similar wilt-inducing properties. In addition, wilt-inducing polysaccharides have been obtained from the endotoxins of two other non-wilt-inducing phytopathogenic bacteria. Hodgson et al. (10) have also demonstrated that wilting may be induced by synthetic polymers, such as Carbowax, and that this type of wilting is caused by a mechanical block. The inherent wilting ability of these polymers is related to their molecular size. One must conclude from this evidence that there is no apparent correlation between the source of a polysaccharide and its wilt-inducing ability.

SUMMARY

Certain toxic complexes may be prepared from three phytopathogenic non-wilt-inducing bacteria, *Agrobacterium tumefaciens*, *Erwinia carotovora*, and *Xanthomonas phaseoli*. These toxic complexes and their polysaccharide components can wilt sunflower and tomato stem cuttings. The source of these polysaccharides and their wilt-inducing capacities apparently are not correlated.

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PHYSIOLOGIC SPECIALIZATION IN UROCYSTIS TRITICI KÖERN.[1]

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(Accepted for publication May 1, 1951)

Flag smut of wheat (*Urocystis tritici* Köern.) occurs in almost all the wheat-growing countries of the world. Verwoerd[2] obtained no evidence of physiologic specialization among several collections of smut tested in South Africa, including a collection from the United States. Yu, Hwang, and Tsiang,[3] on the other hand, described five races of the fungus in China, basing the identity of the races on the reactions of ten wheat varieties during 4 years' tests. Later Yu, Wang, and Fang[4] differentiated 12 physiological races in China on the basis of pathogenicity on four varieties of common wheat and one poulard wheat. In the United States, Holton and Johnson[5] differentiated a collection from Kansas and one from Washington as two races on the basis of the reactions of two selections of an Oro × Federation cross.

Eleven collections of *Urocystis tritici* from six different countries (Table 1) were compared in the present work. Twelve varieties of wheat were inoculated with each smut collection. Four wheat varieties were from the United States and had been used by Holton and Johnson in their work: Federation, Oro × Federation-1, Oro × Federation-26, and Oro × Federation-38. Six were from China: Tsing Hua No. 1932, Nanking No. 716, Ngochen, Grassland, Tsing Hua No. 3929, and a nameless variety that was resistant to the races of flag smut in southern China. Two varieties, Punjab T.9 and 1 P.80-5, were from Pakistan.

Viability of smut spores was tested before any inoculations were made. Only 40 per cent of the spores in the two collections from Pakistan germinated, probably because the smut had been collected more than one year previously. From 70 to 90 per cent of the spores in the other collections germinated. Seed of all the wheat varieties was disinfected with 1:400 formaldehyde solution (with 2 hours' covering) before any inoculations were made.

[1] The author is highly indebted to Professor W. Brown, F.R.S., Head of the Botany Department, Imperial College of Science and Technology, London, for his inspiring guidance and valuable criticism throughout the course of this work, and to donors of the material for their kind cooperation.

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TABLE 1.—*Collections of flag smut*

Collection No.	Designation and origin of collection	Donor
1	Kansas, U. S. (race 1)	A. G. Johnson, U. S. Department of Agriculture
2	Washington State, U. S. (race 2)	do
3	Firenze, Italy	Ministry of Agriculture, Italy
4	Firenze, Italy	do
5	Sydney, Australia	W. L. Waterhouse, University of Sydney
6	Yankang, Chekiang, China (race not known)	T. H. Shen, National Agricultural Research Bureau, Shaolingwei, Nanking
7	Wukung, Shensi, China (probably Chinese race 2)	do
8	Suining, Szechwan, China (probably Chinese race 3)	do
9	Cyprus	Director of Agriculture, Cyprus
10	Lyallpur, Pakistan	A. Sattar, Punjab Agricultural College and Research Institute, Lyallpur
11	Ferozepur, Pakistan	do

Seed and smut spores were shaken together in a glass flask or Petri dish until the seed was thoroughly covered with spores. Seed was sown 3 in. deep in soil with moisture content adjusted to 50 per cent of saturation, in tin pots or wooden boxes; these were covered with thick cardboards until

TABLE 2.—*Percentages of smutted plants in wheat varieties inoculated with 11 collections of Urocystis tritici*

Host[a] and sowing date	Infection percentages with smut collections										
	1	2	3	4	5	6	7	8	9	10	11
Federation											
June 15, 1947	58	79	62	54	76	0	0	0	0	0	0
Oct. 19, 1947[b]	45	....	49	....	56	....	0	0	0	0	....
Oro × Federation-1											
Mar. 10, 1948	9	36	25	57	25	0	0	0	0	0	0
Oro × Federation-26											
Mar. 10, 1948	....	....	17	....	14	0	0	0	0	0	0
Oro × Federation-38											
June 15, 1947	0	35	68	58	80	0	0	0	0	0	0
Oct. 19, 1947[b]	0	48	63	....	76	0	....	....	0	....	....
Mar. 10, 1948	0	24	43	39	11	0	0	0	0	0	0
Nameless Chinese wheat											
June 15, 1947	0	30	31	28	24	0	0	0	0	0	0
Oct. 19, 1947[b]	0	43	....	21	26	....	0	0	....	0	0
Mar. 10, 1948	0	27	22	24	14	0	0	0	0	0	0
Punjab T.9											
June 15, 1947	0	5	7	18	7	0	0	0	0	51	46
Oct. 19, 1947[b]	....	....	10	....	....	0	....	....	0	33	....
Mar. 10, 1948	0	0	0	0	0	0	0	0	0	10	14
1 P. 80-5											
June 15, 1947	0	2	0	0	6	0	0	0	0	0	0

[a] Varieties T.H.1932, No. 716, T.H.3929, Ngochen, and Grassland were inoculated and sown on March 10, 1948, but flag smut tests were not reliable because of very poor germination.

[b] Some varieties in the sowings of October 19 were so severely attacked by powdery mildew that flag smut notes could not be taken.

the seeds germinated. Soil temperature during the pre-emergence period was between 15° and 20° C. Three sowings were made in the greenhouse: June 15 and October 19, 1947, and March 10, 1948.

The plants were grown and observed until heading time and smutted plants were removed as soon as they appeared. A single leaf blade with smut infection was sufficient for considering the plant smutted. Smut percentages (Table 2) were based on counts of 80 to 90 plants of each variety inoculated with a single smut collection.

DISCUSSION OF RESULTS

On the basis of varietal reaction (Table 2) the races under test can be arranged into four groups:

Group 1—Collection No. 1: U. S. race 1.

Group 2—Collections No. 2, 3, 4, and 5: U. S. race 2, two Italian collections, and one Australian collection.

Group 3—Collections No. 6, 7, 8, and 9: three Chinese and one Cyprus collection.

Group 4—Collections No. 10 and 11, both from Pakistan.

The two American collections (one from Kansas and the other from Washington State) have proved to be two distinct races on the basis of the reactions of two selections of an Oro × Federation cross. The author's results are similar to those reported by Holton and Johnson.

The two Italian collections and the one Australian collection are similar to the U. S. collection from Washington State (which constitutes U. S. race 2) because all four infect the same wheat varieties, namely, Federation, Oro × Federation, two American differentials (Oro × Federation-26, Oro × Federation-38), and the nameless Chinese variety. It can be concluded further that these four collections constituting U. S. race 2 are different from the physiologic forms of flag smut occurring in southern China, because these four collections have also infected the nameless Chinese variety, which is resistant to the latter as reported by T. F. Yu, Plant Pathologist, Peiping, China, the donor of this variety.

The Cyprus collection resembles the three collections from China as none of these four (one Cyprus and three Chinese) could infect any wheat variety under trial even though spores were viable in all cases and the temperature was favorable for their germination. It appears that Chinese collections 2 and 3, according to their places of collection as given in table 1 and in the paper by Yu, Hwang, and Tsiang, constitute Chinese races 2 and 3 respectively. It therefore remains to be determined whether the Cyprus collection really resembles any one of these three Chinese collections or differs from them.

The two collections from Pakistan are identical and on the basis of their varietal reaction they constitute a separate race.

It is very unfortunate that, because of very poor germination, the reactions of Chinese differentials to all the flag smut collections could not be

determined. Furthermore, the smut collections from China could not be compared and correlated with those from other countries.

However, from the results it is clear that the two Italian collections and the one Australian collection are the same as U. S. race 2. It is also definite that other collections—three from China, one from Cyprus, and two from Pakistan—do not resemble either U. S. race 1 or U. S. race 2.

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A COMPARATIVE STUDY OF THE VIRUSES CAUSING WISCONSIN PEA STUNT AND RED CLOVER VEIN MOSAIC[1]

D. J. HAGEDORN AND E. W. HANSON[2]

(Accepted for publication May 1, 1951)

In 1937, Osborn[3] reported a virus disease of red clover (*Trifolium pratense* L.) which caused yellowing of leaf veins in this host and which he consequently designated as the vein-mosaic virus of red clover. Hagedorn and Walker[4] in 1949 described a virus disease of pea (*Pisum sativum* L.) widely distributed in Wisconsin, and called it Wisconsin pea stunt: plants were severely stunted, and rosetted apical foliage showed vein chlorosis. Red clover was susceptible but had only faint veinclearing on leaves of diseased plants. The conspicuous vein chlorosis illustrated by Osborn was not observed.

On May 14, 1949, several red clover plants were inoculated with the Wisconsin pea stunt virus isolate used by Hagedorn and Walker and were placed in an insect-proof cage; 8 weeks later, three of these plants had very conspicuous vein chlorosis. Evidently sufficient time had elapsed or the correct environmental conditions had been attained for the development of this symptom. Inoculation of healthy peas with the extracted sap from these diseased red clover plants produced typical Wisconsin pea stunt. This conspicuous leaf-vein chlorosis of red clover, plus the known symptoms on other hosts, suggested that the Wisconsin pea stunt virus might be the same as that earlier described by Osborn. The comparative studies reported in this paper were made to answer this question.

MATERIALS AND METHODS

Since Osborn's original isolate of the red clover vein-mosaic virus was no longer available, 14 red clover plants with typical vein-mosaic symptoms, from six locations in Wisconsin, were used as sources of inoculum. The virus contained in each of these plants produced typical symptoms of Wisconsin pea stunt when transferred to Perfected Wales pea. In the preliminary studies, all 14 isolates appeared to be similar to that described by Osborn and hence only four of them were retained for further study: Nos. 101, 253, 301, and 313. Eventually only one of these, 101, was chosen for more detailed comparisons with the original isolate of the Wisconsin pea stunt virus.

Techniques were essentially the same as those used by Hagedorn and

[1] Published with the approval of the Director, Wisconsin Agricultural Experiment Station. Cooperative investigations between the Wisconsin Agricultural Experiment Station and the Division of Forage Crops and Diseases, U. S. Department of Agriculture.

[2] The writers wish to express their appreciation to Mr. Eugene Herrling for making the photographs used in this paper.

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Walker. All plants were inoculated when young, 3–5 in. high. Inoculated but symptomless plants were observed for at least 90 days and, before they were discarded, inoculations were made from them to healthy peas and red clover to determine whether or not any of the plants contained the virus.

EXPERIMENTAL RESULTS

Symptoms. When plants of Perfected Wales pea were inoculated with either the Wisconsin pea stunt virus or any of the red clover vein-mosaic virus isolates, very similar symptoms were produced (Fig. 1). At 20° C., incubation for about 12 days was necessary before the appearance of first symptoms—plant stunt and inward curling of apical leaves. Shortly there-

FIG. 1. Symptoms on Perfected Wales peas inoculated with five virus isolates. Left to right: healthy check, and plants inoculated with the Wisconsin pea stunt virus and with isolates 101, 253, 301, and 313 of the red clover vein-mosaic virus, respectively.

after the stems had a tannish discoloration and veins in the apical foliage were yellowed. Typical malformation of apical leaves into a tight rosette and stipules with chlorotic veins were noted about 20 days after inoculation. Owing largely to greatly shortened internodes, these malformed plants were frequently only half as tall as normal plants. Diseased plants died or were severely wilted 26 days after inoculation, but axillary bud growth sometimes continued for about a week after the main stem had died.

The first symptom on inoculated red clover plants was a faint veinclearing of leaves. At 20° C. this symptom appeared 20 days after inoculation with some isolates; with others, particularly the Wisconsin pea stunt virus, it sometimes did not appear until after a month or more. However, all of the isolates eventually produced this symptom. The faint clearing of veins gradually developed into a very conspicuous chlorosis (Fig. 2); in some

cases the veins and tissue immediately adjacent were almost white. There was little or no stunting of inoculated plants.

Symptoms on alsike clover, white clover, and white sweet clover were very similar and there was little apparent injury to any of these hosts in the greenhouse. Usually, symptoms appeared in alsike and white sweet clover in 3 or 4 weeks but were not distinct in white clover for at least 60 days. Crimson clover developed vein chlorosis and was severely stunted, and usually the infected plants died.

Broad beans (*Vicia faba* L.) developed veinclearing of apical leaves, about 30 days after inoculation, and later a slight distortion of the tissue on

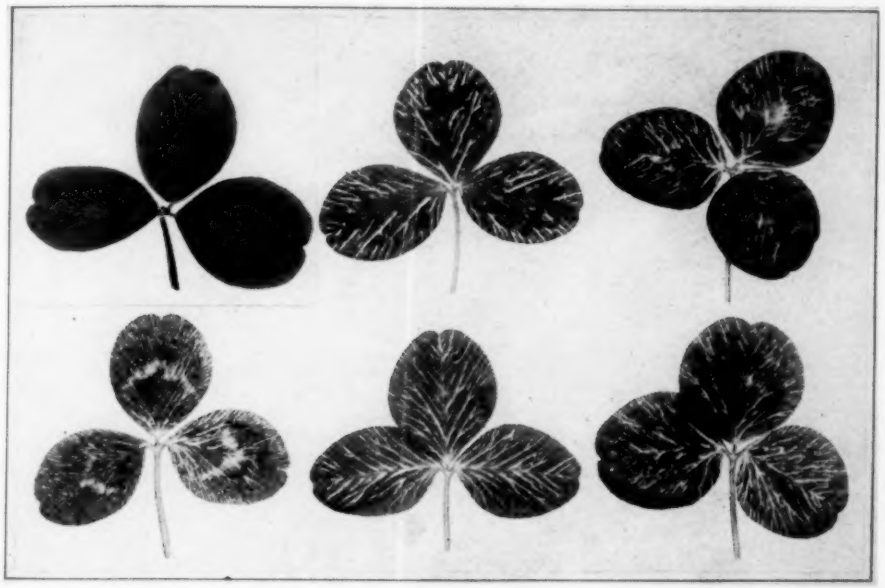

FIG. 2. Symptoms on Wisconsin Mildew-Resistant red clover inoculated with five virus isolates. Top row, left to right: healthy check, and leaves from plants inoculated with the Wisconsin pea stunt virus and isolate 101 of the red clover vein-mosaic virus. Bottom row, leaves from plants inoculated with isolates 301, 253, and 313 of the red clover vein-mosaic virus, respectively.

the under surface of infected leaves which gave the appearance of whitish bands along the veins as described by Osborn. Upper leaves were reduced in size and their margins tended to curl inward. Infected leaves were commonly narrower than corresponding leaves on healthy plants. Many diseased plants became severely stunted; some were 10 to 20 in. shorter than normal. Diseased plants commonly wilted and died back.

Typical symptoms on susceptible host plants are indicated in figure 3.

Effect of air temperature on symptoms. The first of two experiments with red clover was begun on February 3 and the second April 1, 1950, in a series of controlled-temperature greenhouses at 16°, 20°, 24°, and 28° C. respectively. Artificial light was not used nor was any attempt made to control the length of day. Five virus isolates were used, namely, the Wis-

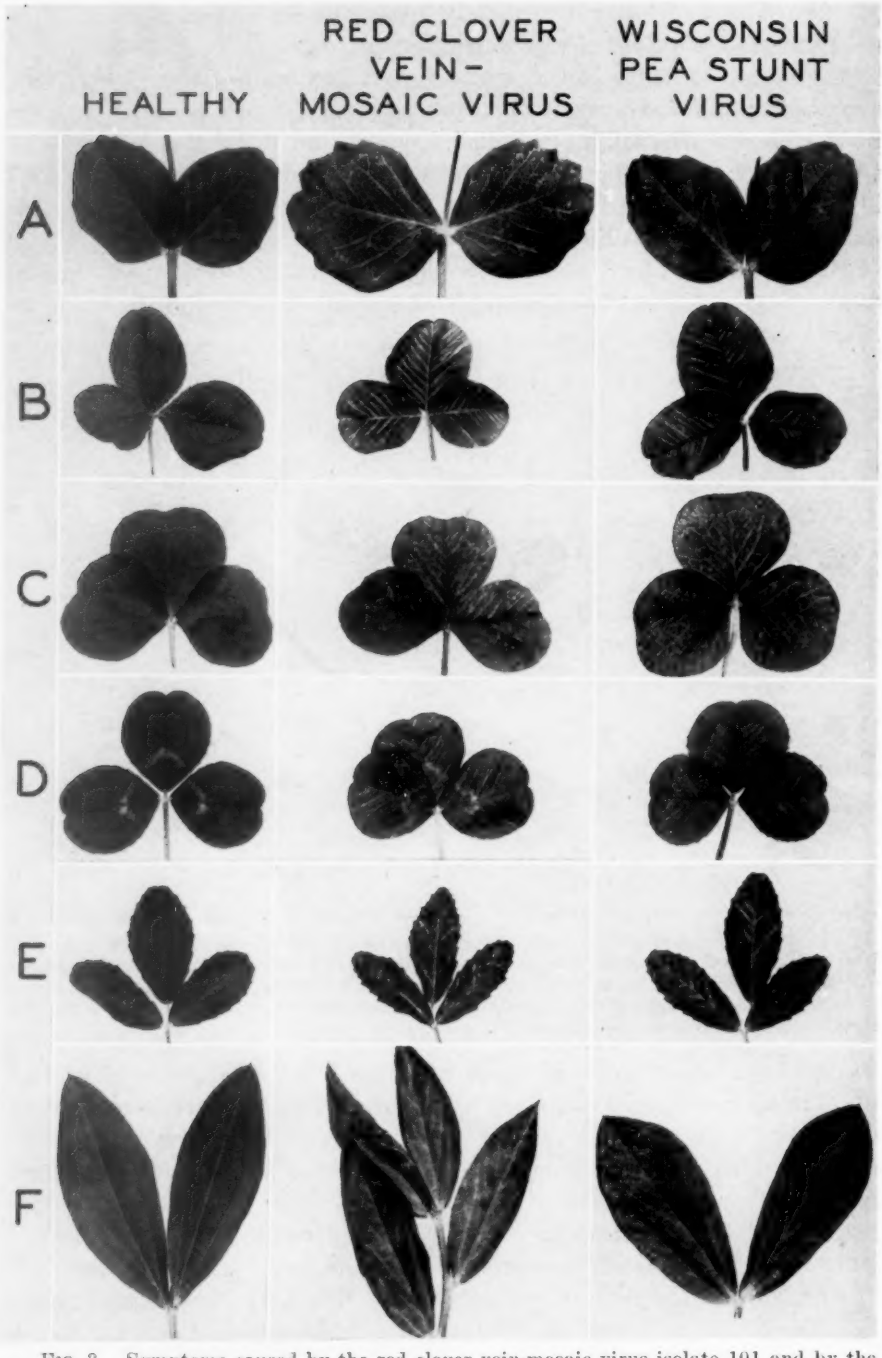

FIG. 3. Symptoms caused by the red clover vein-mosaic virus isolate 101 and by the Wisconsin pea-stunt virus on six leguminous hosts. A, garden pea; B, alsike clover; C, crimson clover; D, white clover; E, white sweet clover; F, broad bean.

consin pea stunt virus and four isolates (101, 253, 301, and 313) of the red clover vein-mosaic virus. In each experiment, 10 plants of red clover were inoculated with each isolate for each temperature. Inoculum was increased on Perfected Wales pea. Prior to inoculation all plants were kept at 20° C.; immediately after inoculation they were placed in the greenhouses at temperatures indicated and observations were recorded every 4 days. The first trial lasted 45 days and the second, 58 days.

In these two experiments the Wisconsin pea stunt isolate caused no symptoms at 28° C. but all other isolates caused good symptoms from 16° to 28° C. On the average 24° C. was perhaps the most favorable temperature for the disease, although, with the one exception noted, there was little difference between 24° and 28° C. in severity or rate of symptom development. Isolate 253 caused symptoms first at the lower temperatures, but its subsequent development was most rapid at 24° and 28° C. In general, disease development was slowest at 16° C.

TABLE 1.—*Comparative host range of the Wisconsin pea stunt virus and isolate 101 of the red clover vein-mosaic virus*

Hosts tested	Diseased plants/Inoculated plants	
	Wisconsin pea stunt virus	Red clover vein-mosaic virus
Lathyrus odoratus L.	12/26	7/24
Melilotus alba Desr.	6/30	27/51
Pisum sativum L.	68/95	74/95
Trifolium hybridum L.	10/34	14/38
T. incarnatum L.	5/44	9/49
T. pratense L.	31/96	39/90
T. repens L.	5/50	6/46
Vicia faba L.	29/60	23/57
Medicago sativa L.	0/40	0/50
Phaseolus vulgaris L.	0/39	0/47
Soja max (L.) Piper	0/22	0/21
Vigna sinensis Endl.	0/31	0/29
Cucumis sativus L.	0/56	0/52
Lycopersicon esculentum Mill.	0/28	0/28
Nicotiana tabacum L.	0/29	0/29

Although there were minor differences between isolates, as indicated by the severity and rate of disease development, the overall performance was much the same. General symptoms were strikingly similar; first vein-clearing, then the progressive change to vein chlorosis.

Host range. After preliminary studies had indicated that several isolates from red clover had the same host range, isolate 101 was chosen for more intensive comparison with the Wisconsin pea stunt virus. Fifteen species from three families were tested at least two or three times. Perfected Wales pea was the susceptible check. The results are in table 1.

The two viruses had an identical host range. Both were limited to the Leguminosae. Susceptible plants were garden pea, sweet pea, crimson clover, red clover, alsike clover, white clover, white sweet clover, and broad

bean. Plants not infected were alfalfa, garden bean, soybean, cowpea, cucumber, tomato, and tobacco. The infective viruses were recovered readily from susceptible plants, but attempts to recover them from species showing no symptoms were unsuccessful.

Pea varietal reaction. To determine whether the two isolates could be distinguished on the basis of their ability to attack garden pea, 10 pea varieties were inoculated with each of the isolates. In two separate trials similar results were obtained (Table 2). All of the pea varieties studied were susceptible to both isolates. Moreover, the symptoms produced by the two isolates were indistinguishable.

Physical properties. The Wisconsin pea stunt virus and red clover vein-mosaic isolate 101 were studied in three different trials in which Perfected Wales pea was used as the source of inoculum and as the test plant.

TABLE 2.—*Reactions of 10 varieties of pea to the Wisconsin pea stunt virus and isolate 101 of the red clover vein-mosaic virus*

Variety	Diseased plants/Inoculated plants	
	Wisconsin pea stunt virus	Red clover vein-mosaic virus
Alaska	10/24	11/24
Alderman	11/15	18/23
Canner King	6/13	6/12
Delwiche Commando	19/29	21/31
Merit	10/22	19/25
Perfected Wales	11/25	15/23
Pride	6/17	9/15
Thomas Laxton	7/11	12/16
Wisconsin Early Sweet	15/27	16/24
Wisconsin Perfection	11/15	18/21

In each test for tolerance to dilution, both viruses were still infective at 1 : 1000. In one trial one plant became infected at 1 : 10,000 but no infection was ever obtained at 1 : 100,000. When aged *in vitro* at 20° C., both viruses were usually inactivated in 2 days but occasionally 3 days were required. The thermal inactivation point was 60° C. for 10 min.

Insect transmission. Healthy pea aphids (*Illinoia pisi* (Kalt.)) were allowed to feed on diseased pea plants for 2 days, placed on healthy peas for a like period, and then killed with an aphicide. Forty per cent infection resulted when the Wisconsin pea stunt virus was studied in this manner, and 66 per cent of the plants tested with isolate 101 of the red clover vein-mosaic virus developed typical disease symptoms. Thus the pea aphid transmitted both viruses.

DISCUSSION AND SUMMARY

The Wisconsin pea stunt disease described by Hagedorn and Walker in 1949 appears to be caused by the same virus that incites red clover vein-

mosaic. Hagedorn and Hanson[5] have suggested this in a preliminary report. The causal viruses had an identical host range (leguminous plants only) and produced similar symptoms on these hosts. Minor differences in some experiments in rate of symptom development, response to temperature, etc., were no greater than those sometimes observed between different isolates of the red clover vein-mosaic virus.

Hagedorn and Walker did not obtain typical symptoms on red clover or infection on white clover. This is quite understandable since, in the present studies, the Wisconsin pea stunt virus had a longer incubation period than any of the isolates of red clover vein-mosaic virus studied. This was especially true on white clover which required at least 60 days for symptom expression. However, symptoms observed eventually in these studies, on the various hosts inoculated with isolate 101 of the red clover vein-mosaic virus and the Wisconsin pea stunt virus, were very similar or identical to those reported by Osborn.

In addition to the striking similarity of symptoms and host range, each of the two viruses studied in detail was capable of infecting all of the 10 pea varieties tested. Among these varieties were Alderman and Perfection which Osborn reported to be susceptible to his red clover vein-mosaic virus. Physical properties and insect transmissibility were also essentially alike.

The definite and impressive similarities existing between the Wisconsin pea stunt virus and isolate 101 of the red clover vein-mosaic virus indicate that they are probably identical.

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[5] Hagedorn, D. J., and E. W. Hanson. The relationship between Wisconsin pea stunt and red clover vein-mosaic. (Abstr.) Phytopath. 41: 15. 1951.

EXTENT OF TRANSMISSION OF CERTAIN CABBAGE PATHOGENS BY SEED GROWN IN WESTERN WASHINGTON[1]

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(Accepted for publication May 4, 1951)

The cabbage (*Brassica oleracea* var. *capitata* L.) seed industry in this country became located in the Puget Sound area primarily because it was found that seed grown there was free of the organisms of blackleg (*Phoma lingam* (Fr.) Desm.) and black rot (*Xanthomonas campestris* (Pam.) Dowson). Walker (9) developed a hot-water treatment to rid infected seed of these organisms, but also showed that the diseases were effectively controlled by growing seed in western Washington (8, 10). Thus, for many years the cardinal recommendation for control of these diseases has been the use of Puget Sound seed. In 1946 Pound (4) reported the occurrence of a variant of *Phoma lingam* in the Puget Sound area and, while he did not demonstrate that it was seed-borne in cabbage, he later (5) pointed out the possibility of this mildly pathogenic strain of *P. lingam* giving rise to blackleg in eastern areas through seed transmission; subsequent surveys in Wisconsin cabbage fields showed that the strain is being so transmitted, but its effects are so mild that it is of little importance. However, in 1947 an epidemic of cabbage blackleg in Wisconsin, in Ohio, and in parts of New York and certain southern States was traced to virulent forms of *P. lingam* carried in cabbage seed grown in the Puget Sound area in 1946 (7). To determine the extent of this development and the possibility of its continued recurrence, cabbage seed lots representing the harvests of 1946, 1947, 1948, 1949, and 1950 have been assayed for infection with *P. lingam* and other organisms pathogenic to cabbage.

METHODS AND MATERIALS

Immediately after harvests, ½-lb. samples of seed, representing different seed fields, were obtained from commercial sources. The fields sampled were representative of the seed-growing area as a whole, with which the senior author is well acquainted. Seeds were surface-sterilized by a 10-min. soaking in a 1:1000 dilution of mercuric chloride containing 20 per cent ethyl alcohol, rinsed in distilled water, and plated out on potato-dextrose agar, 25 seeds per plate. As *Phoma* and yellow bacterial colonies arose they were transferred to test tubes for cultural study and for greenhouse pathogenicity tests. Plates were not discarded until 30 days after plating to give ample time for detection of the slow-growing pathogen of ring spot (*Mycosphaerella brassicicola* (Duby.) Lindau). For comparison purposes

[1] Published with approval of the Director of the Wisconsin Agricultural Experiment Station and the Chief of the Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture.

a lot of cabbage seed which had been produced by plants artificially inoculated with a virulent strain of *P. lingam* was included in most tests. In many tests this lot of seed had 15 to 20 per cent infection.

The laboratory tests were paralleled by greenhouse plantings of un-

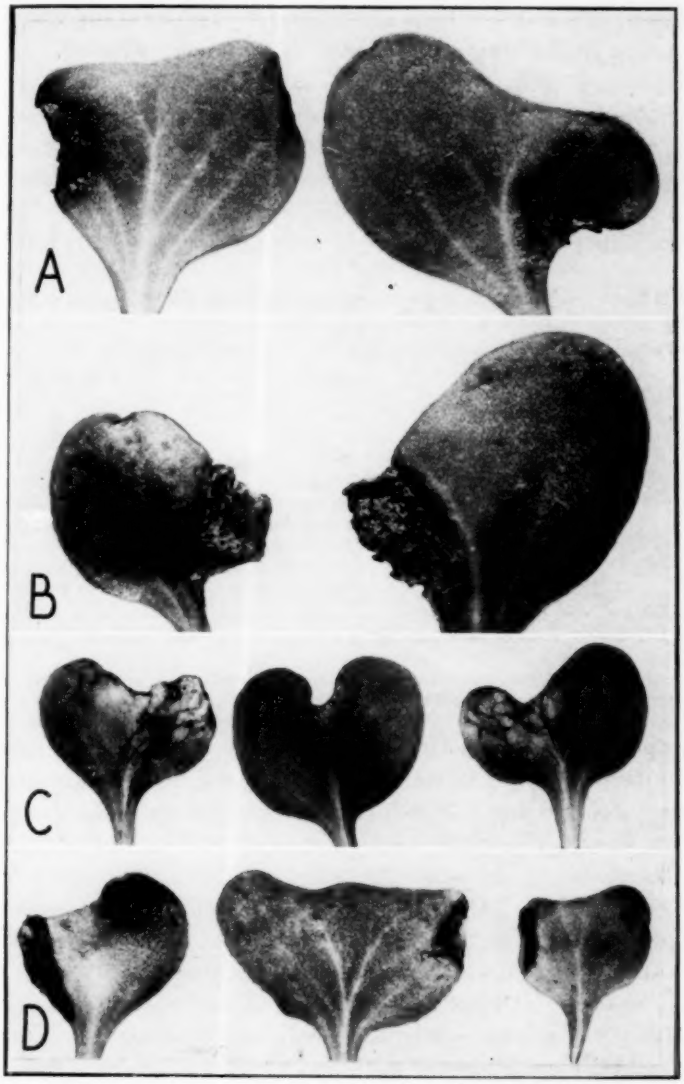

FIG. 1. Cotyledonary lesions of cabbage resulting from seed infection with (A) the Puget Sound strain and (B) an eastern strain of *Phoma lingam*, (C) *Xanthomonas campestris*, and (D) *Alternaria brassicicola*. A and B are enlarged to show greater pycnidium development by the eastern strains of *P. lingam*.

treated seed in sterile quartz sand and in compost soil; individual crocks or pans were used to prevent contamination of one lot of seed by another.

Cotyledonary symptoms resulting from seed infected with *P. lingam*, *Alternaria brassicicola*, and *Xanthomonas campestris* are in figure 1.

RESULTS

1946 Harvest. Of 15 lots of 1946 seed tested, four carried *Phoma lingam* in amounts ranging from 1 to 4 infected seeds per 600 tested. Seed from plants having 15 to 20 per cent infection resulting from artificial inoculation with a virulent form of *P. lingam* yielded 96 colonies of *Phoma* per 600 seeds tested. Isolates (Table 1) identical with the mild Puget Sound strain and with virulent eastern strains (5) were obtained, and isolates resembling the Puget Sound strain in culture also resembled it in virulence, with two exceptions: isolate 10 resembled the Puget Sound strain in culture but eastern strains in pathogenicity; the reverse was true for isolate 11. All seed lots yielding *Phoma* in culture also gave rise to blackleg seedlings in greenhouse plantings. In no instance were the black-rot

TABLE 1.—*Symptom type and cultural characters of Phoma isolates obtained from cabbage seed grown in western Washington in 1946*

Seed lot[a]	Isolates	Symptom type[b] on cabbage	Cultural type on[b] potato-dextrose agar
1	1, 3, and 4	Eastern	Eastern
1	2	Puget Sound	Puget Sound
2	5, 6, and 7	Eastern	Eastern
3	8	Eastern	Eastern
3	9	Puget Sound	Puget Sound
3	10	Eastern	Puget Sound
3	11	Puget Sound	Eastern
4	12	Puget Sound	Puget Sound

[a] Seed lots 1 and 3 were increases of the same stock seed but were grown 200 miles apart.

[b] Eastern refers to the cultural and symptom characters typical of strains occurring in the eastern States; Puget Sound refers to characters of the Puget Sound strain.

and ring-spot organisms isolated in laboratory tests, and no black-rot- or ring-spot-infected seedlings were found in greenhouse plantings. *Alternaria brassicicola* (Schw.) Wiltshire did occur generally, but only in trace amounts.

1947 Harvest. Of 14 seed lots tested in 1947, 800 seeds per lot, two carried *Phoma lingam.* One lot yielded two colonies and the other yielded ten; in both instances the isolates were of the mild Puget Sound strain in both culture and pathogenicity. No virulent isolates were obtained. Three yellow bacterial colonies resembling *Xanthomonas campestris* were obtained from one lot, but controlled greenhouse inoculations showed these to be nonpathogenic. Practically all lots yielded *Alternaria brassicicola* in laboratory platings (ranging from two to ten colonies per lot) and in greenhouse plantings. No colonies of *Mycosphaerella brassicicola* were isolated.

1948 Harvest. May and June of 1948 were very wet and seed were matured and cured under very unfavorable conditions. This was markedly reflected in the seed isolations which showed much more infection with *Phoma* and *Alternaria* than in other years. Of 12 lots tested, six carried *P. lingam* (Table 2). Of the 20 isolates of *P. lingam* obtained, all were identical in culture and pathogenicity with the mild Puget Sound strain.

The control lot of seed yielded 18 per cent seed infection with *P. lingam.* Bacterial isolates were frequent, but none were yellow colonies of *Xanthomonas campestris* type. Pathogenicity tests of representative isolates were negative. Infestations of *Alternaria brassicicola* were very common, but in greenhouse plantings the percentage of seedlings infected with *Alternaria* was surprisingly low. Four definite cases of seed infected with *Sclerotinia sclerotiorum* (Lib.) DBy. were obtained. Lots 1 and 9 were from fields severely affected with *Mycosphaerella brassicicola,* but no isolates of this organism were obtained.

1949 Harvest. Excessive rainfall and floods in the early spring of 1949 caused drastic reduction in acreage of seed fields, in addition to a voluntary reduction due to competition of foreign-grown seed. Only four seed lots were assayed, but 3,000 seeds of each lot were tested. Of these four lots, one carried 1.3 per cent infection with the mild Puget Sound strain of

TABLE 2.—*Results of Petri-plate isolations from cabbage seed lots grown in western Washington in 1948*

Seed lot	Number of seeds, of 800 tested, infected with	
	Phoma lingam	*Alternaria brassicicola*
1	8	45
2, 6, 7, 10, and 11	0	3 to 10
3	1	9
4	1	30
5[a]	7	29
8	2	33
9	0	16
12[a]	1	15

[a] These lots had definite seed infection with *Sclerotinia sclerotiorum.*

Phoma lingam and three carried 1.3, 0.3, and 3.0 per cent *Alternaria brassicicola.* In greenhouse plantings, all the seed lots developed considerable damping-off, and cotyledonary lesions of the two fungi were common. No cases of seed transmission of the black-rot and ring-spot organisms and of the virulent strains of *P. lingam* were found.

1950 Harvest. Six 1950 seed lots were assayed; 500 seeds were tested for each lot. No isolates of *P. lingam, M. brassicicola,* or *X. campestris* were obtained. Only two lots had seed infections by *A. brassicicola,* 0.2 and 1.6 per cent. One lot had 0.2 per cent infection by *Sclerotinia sclerotiorum.*

Rainfall data. In table 3 are rainfall data for April, May, June, and July for each of the 5 years of the test period at Sedro Woolley and Anacortes, points in the eastern and western parts, respectively, of the Skagit Valley in which the cabbage seed lots tested were grown. Rainfall was excessive throughout the valley in June 1946 and May 1948; it was excessive in the eastern part in June 1947 and in the western part in June 1948. June is a much more critical month than May for seed-pod infection. Recorded mean summer temperatures throughout the period were normal.

DISCUSSION

The mild Puget Sound strain of *Phoma lingam* appears to be carried frequently in cabbage seed grown in western Washington. It was isolated in four years of the 5-year test period but never in large amounts. Although it occurred in very small percentages of the seeds tested, a fraction of 1 per cent of seeds infected with virulent strains of *P. lingam* is sufficient to give rise to an epidemic under plant-bed culture. The Puget Sound strain is mild in its effects on cabbage although its symptoms are typical of *P. lingam*. Although infected plants regularly occur in both transplanted and direct-seeded cabbage fields in Wisconsin, losses are negligible; symptoms are those of root rot only (Fig. 2), not sporulating leaf lesions as in the case of virulent eastern forms. The greatest destruction is caused by the damping-off phase in young seedlings. Losses are probably not great

TABLE 3.—*Rainfall data*[a] *for April, May, June, July, 1946–1950, inclusive, for two locations in the seed-growing area*

Station and year	Precipitation (inches) and departure from normal for month indicated			
	April	May	June	July
Anacortes				
1946	1.41/–0.48	0.63/–0.97	3.60[b]/+2.29	0.67/0.0
1947	1.36/–0.53	0.61/–0.99	1.91/+0.60	0.16/–0.51
1948	1.68/–0.21	3.91/+2.31	2.81/+1.50	1.02/+0.35
1949	1.23/–0.66	0.54/–1.06	0.89/–0.42	1.20/+0.53
1950	2.12/+0.23	0.85/–0.75	0.33/–0.98	1.39/+0.72
Sedro Woolley				
1946	3.14/–0.05	0.62/–2.16	6.41[b]/+3.90	0.81/–0.55
1947	4.44/+1.25	1.40/–1.38	4.20/+1.69	0.69/–0.67
1948	2.98/–0.21	4.87/+2.09	3.14/+0.63	1.46/+0.10
1949	3.07/–0.12	0.92/–1.86	2.92/+0.41	1.62/+0.26
1950	4.43/+1.24	3.30/+0.52	1.00/–1.51	1.45/+0.09

[a] Data are from U. S. Weather Bureau Climatological sheets for Washington State.
[b] In this month there were nearly twice as many rainy days as normal and on one day over 2 in. of rain fell.

enough to warrant hot-water treatment of seed or anxiety over the use of Puget Sound seed. Although there is always the possibility of a virulent mutant arising in nature, results to date indicate that mutation toward virulent eastern-type strains is not common (1) and that the 1947 epidemic did not result from such an occurrence.

The blackleg epidemic of 1947 was due to virulent forms of *P. lingam* which were isolated from seed lots of the 1946 harvest, but not from those of any other year. Seed transmission that year may have been due to conditions not often present in the Puget Sound area. The source of the inoculum is not known, but the stock seed from which the infected lots were increased was grown in the eastern half of the United States and was not hot-water-treated before planting. It can be assumed that inoculum was introduced on that stock seed and was perpetuated until the abnormally

wet June of 1946, when it infected seed pods in their most critical stage. This is further indicated by the fact that two lots of seed increased from this stock seed and grown in fields 200 miles apart both carried virulent

FIG. 2. Blackleg symptoms produced in Wisconsin cabbage fields by natural infection with the Puget Sound strain (left) and an eastern strain (right) of *Phoma lingam*. Note close similarity of symptoms.

strains of *P. lingam*. This, if true, should emphasize the need and importance of treating with hot water the stock seed sent into the Puget Sound area for increase. The second seed lot of 1946 harvest which yielded viru-

lent isolates of *P. lingam* came from plants started in the same plant bed as the above lot; they could have been infected during the plant-bed stage. In view of the long period of freedom from blackleg in Puget Sound seed and of the fact that transmission in seed has not recurred in other years (even in the favorable summer of 1948), it seems reasonable to continue to consider Puget Sound seed as relatively disease-free. However, careful watch should be made and seed companies might well consider assaying their seed lots for the blackleg pathogen to avoid other epidemics.

In the three years the senior author spent in the Puget Sound area not a single infection with the black-rot organism was found there (6). Neither has it been found in any laboratory platings or greenhouse plantings of Puget Sound seed. The recent outbreaks of black rot in southern areas, particularly in Florida (2), would, therefore, seem to be attributable to inoculum from some source other than Puget Sound seed. Such sources as foreign-grown seed, residual crop debris, and infested soil should be checked, but since a 10-min. soak in mercuric chloride will eliminate much of the black-rot organism from seed, infected seed might escape detection in the laboratory tests. This is not true, however, of greenhouse plantings. It is reasonable to believe that if the frequent black-rot epidemics of southern areas were due to transmission by Puget Sound seed, such infections would be detected in numerous plantings of the same seed lots in other areas.

In the 5 years of testing seed lots, not a single instance of seed transmission of *Mycosphaerella brassicicola* was obtained. It is known that some of these lots came from fields severely affected with ring spot. In 1944 when the senior author was associated with vegetable seed crop research in the Puget Sound area, a sample of cabbage seed was harvested by hand from pods severely blighted with ring spot and taken from an artificially inoculated plot in which other diseases were absent. Laboratory platings and greenhouse plantings of well-cured seed of this lot consistently failed to show seed transmission of the ring-spot fungus. Over 2000 seeds of this lot were plated out again in 1950 and some 5000 were seeded in the greenhouse, but none yielded *M. brassicicola.* It has been shown that the ring-spot fungus will enter seed but it has never been demonstrated without question that such seed when well cured would give rise to diseased seedlings.

These results with *M. brassicicola* are not in agreement with data recently published by Huber and Gould (3), who reported rather heavy transmission of this pathogen by cabbage seed. However, they simply stated that from plated seed "several fungi, including *M. brassicicola,* developed from the nontreated seed," and their table 2 presented the number of fungus colonies arising from seed, without identification of the fungi involved. In view of the extremely slow growth rate of *M. brassicicola* on potato-dextrose agar, and since the organism does not readily produce spore types by which it can be identified, the writers do not believe accurate identification could be made in 8 days, the period used by Huber and Gould. Nor did these authors point out that cabbage stock seed is not normally pro-

duced in the Puget Sound area and thus is not subject to ringspot infection. Stock seed is normally produced in eastern States or other areas well isolated from the Puget Sound area.

Alternaria brassicicola also was consistently isolated from cabbage seed grown in western Washington. This fungus, however, is a facultative parasite and seldom is important as a seed-borne pathogen. Nontreated seed of lots yielding *Alternaria* in laboratory tests give rise to occasional seedlings showing *Alternaria* lesions, but spread of the pathogen is practically nil in the seed bed in Wisconsin.

Cabbage seeds may apparently be internally infected with mycelium of *Sclerotinia sclerotiorum*. This organism was occasionally isolated from surface-sterilized seed, but since it is a common soil fungus and no conidia are produced to provide secondary spread, its transmission in cabbage seed is of more interest than importance.

SUMMARY

Cabbage seed lots grown in the Puget Sound area of western Washington were assayed to ascertain the extent of seed transmission of the black-rot (*Xanthomonas campestris*), blackleg (*Phoma lingam*), ring-spot (*Mycosphaerella brassicicola*), and black leaf-spot (*Alternaria brassicicola*) organisms. Seeds of the 1946 harvest yielded both eastern-type virulent strains and the mildly pathogenic Puget Sound strain of *P. lingam*. Seed of the 1947, 1948, and 1949 harvests yielded only the Puget Sound strain and the 1950 seed harvest was free of all forms of *P. lingam*. It is suggested that the infection of 1946 seed lots by virulent strains of *Phoma lingam* resulted from the introduction of inoculum on stock seed sent to the area for increase and subsequent seed-pod infection in the abnormally wet June of 1946. In no case was an isolate of *X. campestris* or *M. brassicicola* obtained. *Alternaria brassicicola* was regularly obtained, especially in seed of the 1947 and 1948 harvests. *Sclerotinia sclerotiorum* was occasionally isolated.

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THE ACID TEST FOR PHONY DISEASE OF PEACH

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(Accepted for publication May 11, 1951)

In 1933 Hutchins[2] announced discovery of a chemical test for phony disease of peach. Using a knife or a scalpel, he cut thin cross sections of peach roots (and stems[3]) and immersed them in a test solution prepared by adding 1 to 5 drops of c.p. hydrochloric acid to 25 cc. of absolute methyl alcohol. Readings were made after 3 to 5 min. A positive test was indicated by the appearance of numerous, plainly visible purple dots, well distributed in the wood and in sharp contrast to the whitish or faintly purple background. The entire wood section colored gradually in the solution, finally more or less obscuring the reading. Root sections from normal trees colored slowly and evenly, without dots. Hutchins cautioned against using roots with blemishes, to avoid false readings. Later, Hutchins, Cochran, and Turner[4] reported that phony stem wood may react positively to the acid test, but that it is much less satisfactory than root wood. They also stated that species of *Prunus* other than peach may give somewhat variable reactions, the test being excellent in some, as in *P. davidiana*, for example, and less striking in others.

The red-to-purple dots, which appear in acid-treated wood of phony trees, result from the staining of plugged vessels and thickened walls of neighboring cells, as has been described by Esau.[5] In sections stained with iodine green, some of the dots are plainly visible, without magnification, as dark green dots on a lighter background. Although invisible in freshly cut wood, the larger dots may be visible as faint orange spots in the wood after a few weeks' storage in nonacidified methyl alcohol. Finally, the material that stains appears to be so leached out by methyl alcohol that the sections will no longer give a positive acid test for phony.

The reaction of wood from phony-affected trees to the acid test is somewhat analogous to the development of photographic films in that the "image" is present, but invisible, before treatment; the speed of the reaction depends upon the composition, concentration, and temperature of the reagent; and the reaction will go farther than desirable unless measures are taken to prevent it. For convenience, the reagent employed to bring out the

[1] Plant pathologist, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Fort Valley, Georgia.

[2] Hutchins, Lee M. Identification and control of the phony disease of the peach. Ga. State Entomol. Bul. 78. 1933.

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test will be called the "developer" and the configuration of dots the "image."

Any moderately strong acid will serve as a developer for the phony acid test. Liquid acids (nitric, sulfuric, hydrochloric, formic, phosphoric, phosphorous, sulfurous, lactic, acetic, and propionic) are effective developers without the addition of any other substance. Without dilution, the first three named produce almost instantaneous images (at 25° C.), but overdevelopment follows rapidly. They are effective at high dilutions in alcohol. The images remain readable for a period roughly twice as long as that required for development. In strong solutions these three acids will produce images on the cut ends of roots without sectioning; but the method is not recommended, because faint images will be overlooked. The acids are useful as developers in 1 to 10 per cent solutions in methyl or ethyl alcohol. Undiluted formic, phosphoric, and phosphorous acids produce images in minutes (25° C.), but they are unsatisfactory because the images are not sharp. Sulfurous acid requires hours to produce an image. Lactic, acetic, and propionic acids require 5 to 10 hr., 2 to 3 days, and 3 to 4 days, respectively, to produce discernible images, and much more time is required for full development. Sections left in lactic acid 15 months were not seriously overdeveloped.

Absolute methyl alcohol solutions of trichloroacetic, oxalic, tartaric, salicylic, citric, malic, and succinic acids all give very sharp images. These acids are listed in descending order as to time required for development of an image in a concentrated solution at 25° C.—from seconds for trichloroacetic acid to about a day for succinic acid.

The specific solvent or diluent used greatly affects the time required for development. Acids dissolved in or diluted with methyl alcohol develop images faster than when ethyl alcohol is used. Ethyl alcohol gives a faster reaction than higher molecular weight alcohols. However, hydrochloric acid and oxalic acid are effective in all liquid alcohols tried (methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, secondary-butyl, n-amyl, iso-amyl, and diethylcarbinol). Picric acid is effective in benzene. Many of the acids are effective in acetone, but the image is not sharp and tends to be transitory. The addition of zinc chloride, potassium bromide, or sodium chloride to liquid organic acids or to organic acids in methyl alcohol solutions greatly hastens development, even where no appreciable amount of the halide appears to have dissolved. The addition of water to any developer slows the reaction, and crystalline acids are ineffective when dissolved in water. However, strong inorganic acids may be diluted considerably with water. Nitric acid in a 2 per cent aqueous solution is an effective developer.

The color of the image is related to the strength of the acid used as a developer. Liquid inorganic acids and oxalic acid in methyl alcohol produce purplish-red to purple dots on a faintly red-to-purple background. Tartaric, salicylic, citric, and malic acids in methyl alcohol produce pink-to-

red dots on a faint pink background. Trichloroacetic acid produces orange-to-brick red dots on a yellow-to-orange background. Propionic acid produces yellow dots on a very faint yellow background.

The configuration of dots and all other color in acid-treated wood disappear quickly if the sections are immersed in a concentrated solution of sodium hydroxide in methyl alcohol. Wood sections may be stained and de-stained repeatedly until finally the wood turns light brown.

Although the phony acid test reaction is a reversible color reaction produced by any strong acid and removed by alkali, no wholly satisfactory substitute has been found for methyl alcohol acidulated with hydrochloric acid. However, chemically pure ingredients are unnecessary and the concentration Hutchins recommended gives too slow a reaction at temperatures below 22° C., particularly when fresh stem wood is being tested. When tests are made in large numbers, it is desirable that images be readable in less than a minute. For out-of-door work, 1 part of commercial hydrochloric acid to 4 parts of technical methyl alcohol usually is a satisfactory reagent, but at temperatures below 5° C. 1 min. or more is required for development of images with the undiluted acid. Hydrochloric acid that is not highly diluted should be handled in glass dishes with small surface areas to avoid irritation of the eyes by acid fumes. Constant use of strong solutions of hydrochloric acid in the laboratory is likely to cause irritation of the eyes and rusting of iron instruments. A warm solution (concentrated) of technical oxalic acid in methyl alcohol is satisfactory where speed is desired and fuming is not. Excellent images may be obtained with strong solutions of oxalic, citric, succinic, tartaric, and malic acids in methyl alcohol at 20°–25° C. in stoppered vials in minutes to hours. Lactic acid (85 per cent) gives an excellent image overnight. Trichloroacetic acid in methyl alcohol is a fast developer and gives very sharp images when the concentration is adjusted to give a reading in not less than 1 min. (nearly concentrated solution for 20° C.), but the solution is irritating to the skin. Images that are developed slowly (in not less than 10 min.) may be preserved by transferring the sections to glycerine before full development takes place.

Usually most roots more than 1 cm. in diameter and many of the smaller roots of peach trees showing phony symptoms give a positive acid test for phony. The distribution of stem wood that will give a positive test is less general, and the dots in transverse wood sections require more time for development and are often fewer and smaller, so that examination under a lens or dissecting microscope is necessary for accurate reading of acid treated stem sections. In summer, stem wood of current-season growth is less likely to give a positive phony reaction than is older wood. In all cases the dots are confined to the xylem. Root and stem wood from phony trees of other *Prunus* spp. also give positive phony tests on acid treatment.

Frequently, fresh wood from phony peach trees, particularly stem wood, is a somewhat darker color than wood from healthy trees and may contain

longitudinal streaks of necrotic tissue. These streaks appear in cross section as small spots or flecks, with a configuration similar to that of the colored dots seen in acid-treated wood from phony trees, and stain with acid in a similar manner. Thus, it appears that the flecks in wood from phony trees and the dots that are visible only after acid treatment are stages of the same condition. However, wood sections with flecks are avoided in acid testing for phony disease, because necrotic tissue in peach wood stains quickly with acid and flecks may arise from causes other than phony disease. Longitudinal streaks of necrotic tissue extend from cankers and die-back beyond the point where they are plainly visible and may give false positive readings on acid treatment. Because it is much easier to ascertain absence of blemishes in stems than in roots, there is considerable advantage in using stems for acid testing. Stems 5 to 10 mm. in diameter are most satisfactory from the standpoint of simplicity in procedure, although stems of any size can be used.

The image that appears on acid treatment of wood from phony trees varies from a single dot, too small to be seen plainly without using a lens, to dots so large, numerous, and confluent as to form splotches covering more than half the section. Hutchins originally suggested a minimum of 10 scattered dots for a positive reading. However, fewer dots appear to be significant.[6] Investigations on the diagnostic value of the acid test are continuing.

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[6] KenKnight, Glenn. The acid test for phony disease of peach and its diagnostic value. (Abstr.) Phytopath. 41: 20–21. 1951.

MECHANICAL TRANSMISSION OF CORN STUNT VIRUS TO AN INSECT VECTOR

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(Accepted for publication May 14, 1951)

Kunkel (14) found that in the United States corn stunt virus is transmitted by the leafhopper *Dalbulus maidis* DeL. & Wol. (known formerly as *Baldulus maidis* DeL. & Wol.) (5). The incubation period of the virus in the insect, as reported by Kunkel (15), is at least 14 days. In Mexico this virus is transmitted by the related species of *D. elimatus* Ball (21). Corn stunt has never been transmitted mechanically. The purpose of the present investigation was to find if possible a mechanical means of transmission. Such a finding would facilitate the *in vitro* study of the corn stunt virus.

LITERATURE

The first mechanical transmission of an infectious agent to an insect vector was performed by Weigl (25) who transmitted *Rickettsia prowazekii* da Rocha-Lima to the louse (*Pediculus humanus corporis* DeGeer). In plant pathology the first experiments of this kind were carried out by Storey (24), who transmitted maize streak virus to *Cicadulina mbila* Naude. Storey used viruliferous insects as well as diseased plants as a source of virus. Black (1) transmitted aster-yellows virus mechanically from viruliferous to virus-free individuals of the vector *Macrosteles divisus* Uhler. In later unpublished experiments Black succeeded in artificially transmitting the aster-yellows virus from infected plants to insects. Maramorosch, Brakke, and Black (20) showed that wound-tumor virus can be transmitted by means of injections to a leafhopper, *Agallia constricta* Van Duzee. The three plant viruses thus carried over to insects have not so far been transmitted mechanically to plants. In view of this fact the insect-injection method was tried in the present investigation with corn stunt virus, which also cannot be transmitted mechanically to plants. A preliminary report of the results has been published recently (19).

MATERIALS AND METHODS

The leafhoppers used in the experiments were obtained from stock cultures of nonviruliferous *Dalbulus maidis*, raised on healthy corn plants (*Zea mays* L. var. Yellow Dent) in an insectary. Insects from nonviruliferous stock cultures were included as controls in all tests. None transmitted the corn stunt virus during the course of the work.

Corn plants used for the test were grown from commercial seed in 4-in. pots and caged with insects as soon as the seedlings emerged. Insect cages, 3.5 in. in diameter and 10 in. high, were made of transparent cellulose nitrate (Fig. 1, A) with tops and side openings covered with plastic screen (Lumite 60 × 60 mesh). Large corn plants were caged with lantern globes

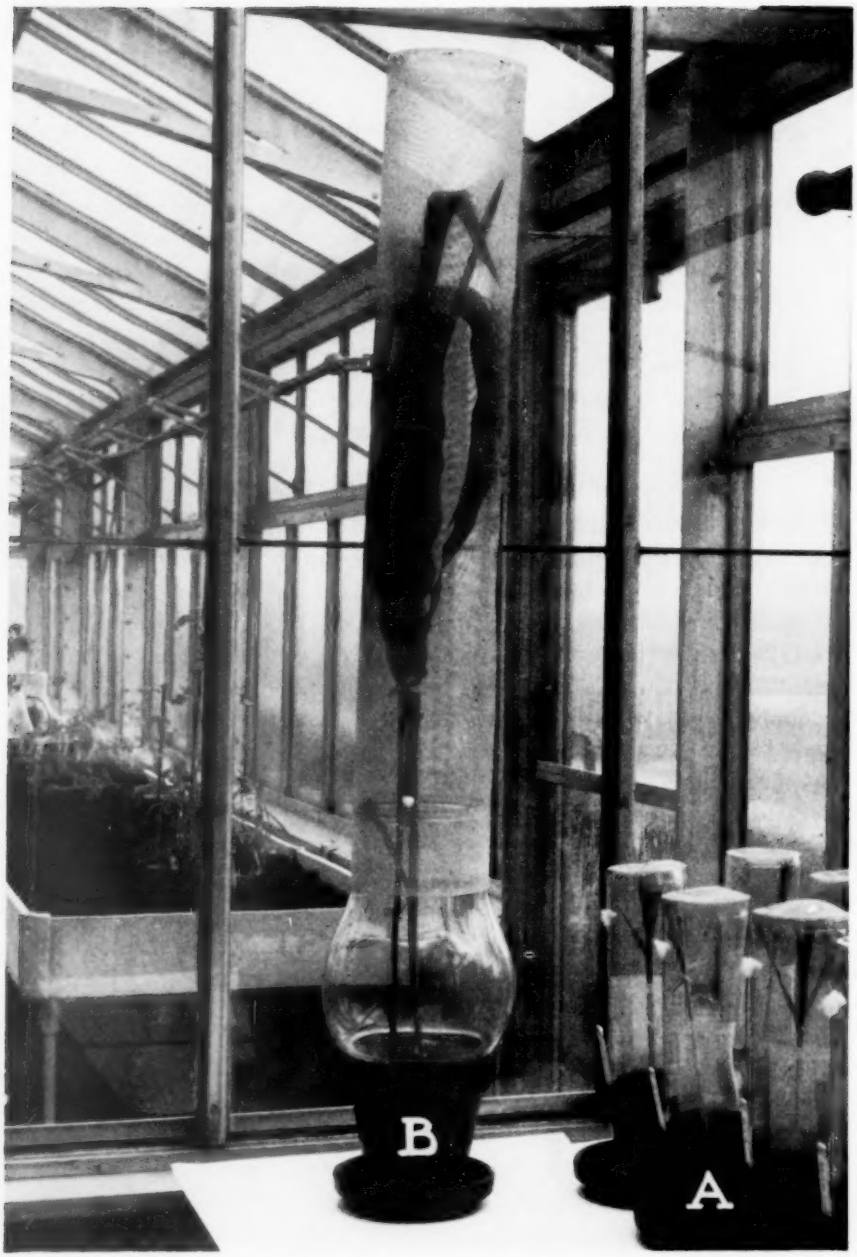

FIG. 1. Insect cages. A. Celluloid cages. B. Lantern globe combined with cylinder of plastic screening. (Photograph by J. A. Carlile.)

combined with 3-ft. cylinders of plastic screen (28 × 28 mesh) and celluloid tops (Fig. 1, B). These cages provided excellent conditions of light and ventilation for the caged plants and insects.

A specially constructed and calibrated microsyringe and 33-gauge hypodermic needles of stainless steel were used for injections of insects.

Nymphs of *Dalbulus maidis* from virus-free stock cultures were placed in a test tube on a portion of a diseased corn leaf for 24 hr. and later caged for 4 weeks on a corn plant in the greenhouse. It was assumed that during that time the incubation period of the acquired virus was completed in the leafhoppers. Fifty of these viruliferous insects were used as a source of inoculum in each of the first three experiments. They were macerated at 0° C. in a 5-cc. TenBroeck tissue grinder, mixed with 99 times their weight of 0.25 M neutral buffered NaCl solution. The suspension was centrifuged at 3000 r.p.m. for 10 min. Amounts of the supernatant fluid varying from 1/1000 to 1/8000 cc. were injected into the abdominal cavities of virus-free adult leafhoppers. Plants were used as a source of virus in the fourth experiment; the juices were diluted 1:100 and centrifuged, and the supernatant used as inoculum in the same manner as described for the insect juice. The insects were held at 0° C. in a coldroom, to immobilize them during injection, and were then slowly warmed to room temperature and caged on healthy corn plants. Noninjected insects from the same lot were set up as controls. The inoculated and the control leafhoppers were tested individually or in colonies of five on fresh plants and transferred at weekly intervals. The test plants were observed for 3 to 4 months in the greenhouse. With each transfer the number of controls was reduced to correspond with the number of surviving injected insects.

EXPERIMENTS AND RESULTS

In the first experiment 100 virus-free *Dalbulus maidis* were injected with a 10^{-2} dilution of juices from 50 viruliferous leafhoppers. Only eight inoculated insects survived longer than 5 weeks.[1] Three of these died after 6 weeks, one after 7 weeks, and one after 10 weeks; no virus transmission was recorded for the five. The first two transmissions (Insects 1 and 2) occurred during the 7th week, the third transmission (Insect 4) during the 8th week. Thus the incubation period of the virus in the injected vectors was at least 6 weeks at greenhouse temperatures of approximately 21° C. (70° F.). An equal number of control insects, transferred at weekly intervals to an equal number of plants, did not cause disease. Of the inoculated insects, No. 1 and No. 4 infected all nine plants on which they fed after completion of the incubation period. Insect No. 2 infected only one plant during the 7th week, and died during the 8th week without infecting the last test plant on which it fed.

In the second experiment, another 100 *Dalbulus maidis* from stock cultures were injected as described above and later caged on a single large corn plant for the first 3 weeks. Insects that survived this period were

[1] The question of whether the low number of surviving injected insects was due to the effect of the virus or due to the wounding, or combinations of the two, cannot be answered at present. Unwounded control leafhoppers always survived much longer than injected ones.

tested on individual corn plants at weekly intervals. The transmission record of the 10 insects that survived 7 weeks or longer indicated that five of them became infective after a minimum incubation period of 6 weeks. Insects No. 1, 2, 3, and 4 transmitted the virus to all test plants as long as they lived. In the case of insect No. 5 the transmission record was probably also uninterrupted, but the test plant used in the 9th week died before disease symptoms could develop. All control insects proved virus-free.

In a third test, 100 injected insects were fed for 2 days on a large corn plant, then 30 survivors were caged in groups of five on healthy young corn plants and transferred after 5 days and later at the usual weekly intervals. In this experiment (Table 1), three colonies became infective and remained so until they died. The minimum incubation periods in these colonies were 6, 7, and 8 weeks, respectively.

TABLE 1.—*Mechanical transmission*[a] *of corn stunt virus to an insect vector*

Colony		Weeks											
		1	2	3	4	5	6	7	8	9	10	11	12
1	a	5	5	4	2	2	2	2	2	1	1	1*	
	b	−	−	−	−	−	−	+	+	+	+	+	
2	a	5	4	3	3	2	2	2	2	2*			
	b	−	−	−	−	−	−	−	+	+			
3	a	5	3	3	2	1	1	1	1	1*			
	b	−	−	−	−	−	−	−	−	+			
4	a	5	4	4	3	2	1	1	1	1	1	1	1
	b	−	−	−	−	−	−	−	−	−	−	−	−
5	a	5	2*										
	b	−	−										
6	a	5	4	4	4	2	1	1*					
	b	−	−	−	−	−	−	−					

[a] For each colony, line a represents number of insect survivors; line b, transmission record (− indicates healthy plant; + indicates diseased plant). A star (*) indicates death of the last insect in a colony. Ten control insects, tested as above on an equal number of plants, proved virus-free.

In the fourth and last experiment, juice from a diseased corn plant was used as a source of virus. A portion of a leaf with severe symptoms of corn stunt was macerated at 0° C. with 99 times its weight of neutral buffered 0.25 M NaCl solution and centrifuged for 10 min. at 3000 r.p.m. Amounts of the supernatant fluid not exceeding 1/8000 cc. were injected into each of 120 virus-free adults of *Dalbulus maidis*. Only five insects survived more than 3 weeks and these were tested individually on corn plants. Two of them transmitted corn stunt to successive plants as long as they lived. The minimum incubation period of the virus was 6 and 7 weeks, respectively. The 20 control insects, tested at weekly intervals on an equal number of plants, proved virus-free.

In the four experiments described above the incubation period of the virus in the infected plants (that is, the time between the first day of infective test feeding and the first day symptoms appeared) varied from 6 weeks to 3 months.

DISCUSSION

The experiments reported here provide a technique by which corn stunt virus can be readily detected in extracts from both insects and plants. The ease with which the virus was transmitted to insects in each of the tests is in striking contrast to the failure of mechanical attempts to transmit it to plants. It is a question why the corn stunt virus, like other plant viruses of the yellows group, is easily transmitted to the vector, but not to the plant. One possible explanation may be that the virus multiplies in the phloem of the host and has to be introduced into a living phloem cell with little or no injury to the latter. This is done without difficulty by the leafhopper's proboscis in a manner that cannot be duplicated at present by mechanical methods. On the other hand, the virus, injected into the vector's abdominal cavity, may be taken up by the blood and carried to structures, as yet unknown, where it may multiply during the incubation period. Although the multiplication has not been experimentally demonstrated for corn stunt virus, its long incubation period and other characteristics place it in the same category as other leafhopper-transmitted yellows viruses having long incubation periods. Experimental evidence for the multiplication of rice stunt virus has been published by Fukushi (6, 7), of aster-yellows virus by Kunkel (12, 13) and by Black (1), of clover club-leaf virus by Black (2). These viruses have multiplied readily in plants and insects, and are therefore really both plant and animal (insect) viruses. Two leafhopper-transmitted viruses, aster-yellows (18) and wound-tumor virus (3), have recently been carried through insects in serial passages. The lengths of incubation periods of these two plant viruses in their vectors were found to vary proportionately with the dose of the injected virus (16, 17). A similar relationship between the dosage of injected virus and the length of incubation period was described for the animal viruses causing rabbit papilloma (4) and mouse encephalomyelitis (8). So far as is known, the yellows viruses cause no apparent insect diseases. In this respect they resemble other plant and animal viruses that cause no noticeable symptoms in their hosts (9, 10, 11, 22, 23).

The insect-inoculation method may prove useful in studies on the properties of plant viruses that have not as yet been transmitted mechanically.

SUMMARY

Corn stunt virus from viruliferous *Dalbulus maidis* was injected, after dilution, into virus-free insects of the same species. Insect-to-insect transmission was successful. In a further experiment, virus in juice from infected plants injected into insects caused the latter to become infective.

Thus a fourth plant virus is added to the list of those that are not yet mechanically transmissible from plant to plant but are mechanically transmissible to insect vectors.

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INDUCED HYBRIDIZATION IN GRAMINICOLOUS SMUT FUNGI. I. USTILAGO HORDEI × U. BULLATA[1]

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(Accepted for publication May 15, 1951)

Interspecific and intergeneric hybridization in the smut fungi, experimentally induced, has been of considerable interest during the past 20 years, both from the standpoint of an understanding of the genetics of this important group of plant pathogens and as further empirical evidence of a means of origin of new species.

Kniep in 1926 (30) considered that he had produced hybrids between several *Ustilago* spp. when he obtained fusions between sporidia of different species. He found that the sporidia of reticulate-spored species would not fuse with those of species having smooth or echinulate spores. Likewise, Bauch in 1934 (3) gave an account of hybridization between bipolar and multipolar species of smut fungi. As with Kniep's "hybrids," Bauch's crosses consisted of sporidial fusions. However, the general concept today is that hybridization has not actually been accomplished unless first-generation hybrid chlamydospores are produced.

Apparently the first instance of induced hybridization of smut fungi was by Kämmerling (29), who in 1929 hybridized *Ustilago longissima* (Schlecht.) Meyen with *Ustilago davisi* Liro (*Ustilago longissima* var. *macrospora* Davis). In 1930 Hanna and Popp (16) first succeeded in crossing the loose smut of oats, *Ustilago avenae* (Pers.) Rostr., with the covered smut of oats, *U. kolleri* Wille. Many additional hybrids between these species have been obtained in subsequent years by Holton (17, 18, 19, 20, 21, 23), Popp and Hanna (34), Hanna and Popp (16), and Utter (43).

Flor in 1931 and 1932 (12, 13) was the first to hybridize *Tilletia* spp. He obtained F_1 chlamydospores of *T. caries* (DC.) Tul. × *T. foetida* (Wallr.) Liro, the common species causing bunt or stinking smut of wheat. Subsequent additional hybrids were obtained by Hanna (15), and Holton (22, 24, 25, 26, 27). Gassner, in 1938 (14) described several cases of spontaneous hybridization between *T. caries* and *T. foetida*. Holton's studies (24) included hybridization of *T. foetida* with the "dwarf bunt" race of *T. caries*.

In 1934, Ruttle (40) produced the first hybrids between the covered smut of barley, *Ustilago hordei* (Pers.) Lagerh., and the black loose smut of barley, *U. nigra* Tapke (*U. avenae*). Additional hybrids were later reported by Allison (1, 2), Hungerford (28), and Bever (4).

Rodenhiser in 1932 and later (35, 36, 37, 38) and Rodenhiser and Barnes (39) reported on experimentally induced hybridization between the two sorghum smuts *Sphacelotheca sorghi* (Link) Clint. and *S. cruenta* (Kühn) Potter.

[1] Scientific Paper No. 1006. Washington Agricultural Experiment Stations, Institute of Agricultural Sciences, The State College of Washington, Pullman.

Vaheeduddin in 1936 and later (44, 46) succeeded in hybridizing *Sphacelotheca cruenta* with *S. reiliana* (Kühn) Clint. In 1935 Tyler and Shumway (42) produced hybrids between *S. sorghi* and *S. reiliana.*

Hybrids between two Panicum smuts, *Sphacelotheca destruens* (Schlecht.) Stevenson & A. G. Johnson and *Sorosporium syntherismae* (Tk.) Farl., were obtained in 1943 by Martin (33). This appears to be the only successful attempt to produce intergeneric hybrids in the smut fungi.

In 1940 Fischer (6) described sporidial fusions between *Ustilago striiformis* (Westend.) Niessl and *U. hordei* and *U. bullata* Berk., and suggested that it might be possible to produce hybrids between these three smut fungi. In 1948, Fischer (10, 11) gave a brief preliminary report on hybridization between *Ustilago hordei* and *U. bullata* and between *U. striiformis* and *U. bullata.* The present paper is an extended account of hybridization between *U. hordei* and *U. bullata.*

MATERIALS AND METHODS

In every reported case of hybridization between species and genera of the smut fungi the two hybridizing species have had a common host. The susceptibility of *Agropyron* and *Elymus* spp. to *Ustilago hordei* (5) and *U. bullata* suggested that these smut species might be induced to hybridize.

All hybrids were obtained by inoculating with paired pedigreed monosporidial cultures of opposite sex of *Ustilago bullata* and *U. hordei.* Fourteen monosporidial lines of *U. bullata,* representing four races, and six of *U. hordei,* representing one race, were used.

The monosporidial lines of both species were paired on 3 per cent water agar in all possible combinations to determine which combinations were compatible. The presence of fused sporidia and the subsequent development of infection hyphae in paired lines were considered as a reliable criterion of sexual reaction inasmuch as fusions and infection hyphae did not occur in selfed monosporidial lines.

In preparing the inoculum an entire Petri plate of 3 per cent water agar was smeared with fresh cultures of two monosporidial lines of opposite sex and of desired parentage. Care was taken to thoroughly mix the sporidia and to spread the mixture evenly over the agar surface.

Seeds of the common host were soaked in 1:240 formaldehyde solution for 1 hr. to free them of any foreign smut, and were then washed in running water for several minutes to remove the formaldehyde. Seeds to be inoculated were first incubated between layers of wet paper toweling until they showed first signs of germination. For inoculation, enough of the hull of each seed was removed to expose the developing embryo, and each seed was placed on the agar so that the young hypocotyl was in direct contact with the agar surface bearing the inoculum. Approximately 50 germinating seeds were inoculated with each combination. The plates were covered and incubated at room temperature until the seedlings were push-

ing against the Petri-dish lid. The inoculated seedlings were then transplanted to benches of soil in the greenhouse where they were allowed to grow to maturity. Inoculations were made with 14 combinations of *Ustilago hordei* × *U. bullata*. A susceptible host was inoculated with each parental monosporidial line.

Two grasses known to be hosts in common for both parent smut species were selected for the hybridization experiment: *Elymus canadensis* L., and *E. glaucus* Buckl. Check plantings of noninoculated seed were made of each of the host species as well as of barley and grasses used in the F_2 inoculations. The smutted heads representing the F_1 sori were carefully harvested and stored in individual packets.

As will be explained below, inoculations with the F_1 hybrids had to be performed with chlamydospores. The seed was inoculated with aqueous suspensions of these spores, using the partial vacuum method, and planted in flats of sterilized soil in the greenhouse immediately after.

RESULTS

Nine of the fourteen attempts to cross *Ustilago bullata* and *U. hordei* were successful, yielding varying amounts of F_1 sori on *Elymus canadensis* and *E. glaucus*. Infection ranged from 22 to 45 per cent, by head count.

Inasmuch as both *U. bullata* and *U. hordei* are head smuts, there were no contrasting macroscopic or symptom characters to study. Microscopically, however, there were three contrasting chlamydospore characters involved in these hybridization attempts: spores of *Ustilago hordei* are smooth and distinctly lighter on one side, and measure 5–8 μ in diameter; those of *U. bullata* are verrucose, the wall is uniformly dark, and in some races spore diameter is as much as 12 μ.

The F_1 hybrids involve three physiologic races of *Ustilago bullata:* Race 1 (one hybrid), common on species of *Agropyron, Elymus,* and *Hordeum;* Race 2 (four hybrids), common on cheat or downy brome, *Bromus tectorum* L., but will attack some strains of *Elymus glaucus;* and Race 12[2] (four hybrids), common on *Bromus purgans* L. and other *Bromus* spp. but will attack also some lines of *Elymus canadensis*. The *U. hordei* parent represented a spontaneous occurrence of this species on *E. canadensis* (8).

In every instance the F_1 chlamydospores were at least minutely echinulate and usually rather distinctly so, thus proving the partial dominance of the verrucose spore wall in the *Ustilago bullata* parent. Likewise, in every case the F_1 chlamydospores were lighter-colored on two sides. This indicated the dominance of the character in *U. hordei* wherein the spores are lighter-colored on one side, and probably a gene in the *U. bullata* parent for the same character which is not expressed unless combined with genic material from another source. The size of the F_1 chlamydospores was intermediate between the two parents (Figs. 1–4).

[2] Unpublished data on new races of *Ustilago bullata*.

Attempts to obtain pedigreed monosporidial cultures from the F_1 chlamydospores were uniformly unsuccessful. Germination was induced readily on potato-dextrose agar, but sooner or later lysis would occur in the promycelium and sporidia much like that described by Laskaris (31, 32) in intraspecific hybrids in *Sphacelotheca reiliana*. Since no monosporidial cultures were obtained it was necessary to use F_1 chlamydospores as inoculum to obtain the F_2 generation. Whichever of the two species of *Elymus*, *E. canadensis* and *E. glaucus*, proved to be a compatible host for the F_1 generation was used also as the host for the F_2 generation. The seeds were inoculated with aqueous suspensions of the F_1 spores and planted in flats of steam-sterilized soil in the greenhouse. The resulting seedlings were transplanted to benches in the greenhouse and allowed to grow to maturity. Six of the nine F_1 hybrids produced a second generation. Three of the F_2's involved Race 2 and three Race 12 of *Ustilago bullata*. These will be described in some detail.

No smut appeared in the rows planted with noninoculated seed nor where seed had been inoculated with monosporidial cultures of either species.

Hybrid 749.[3] The *Ustilago bullata* parent of Hybrid 749 is Race 2, represented by collection M-A as already described (7). This race is common on cheat or downy brome, *Bromus tectorum*, and will not infect barley. The smooth exospore, lighter-colored on one side, of the *U. hordei* parent and the granular verrucose exospore, with uniformly dark wall, of the *U. bullata* parent provided widely contrasting morphological characters for inheritance studies.

As already indicated, the chlamydospores of the F_1 generation, which were produced on *Elymus glaucus* as the host common to both parents, were neither smooth nor verrucose. They were echinulate, which condition might be regarded as an intermediate expression of the extremes represented by the parents. Furthermore, the F_1 chlamydospores were distinctly lighter-colored on two sides and the echinulations were especially noticeable on the clear sides (Fig. 1). This condition can hardly be regarded as intermediate between the two parents. Sometimes the spores of some races of *U. bullata* appear to have a dark equatorial band. Perhaps this character, combined with that of *U. hordei* wherein the spores are lighter on one side, could produce the striking characteristic of distinctly lighter-colored (almost hyaline) areas on two sides.

When the F_1 spores were used as inoculum on *Elymus glaucus*, *Bromus tectorum*, and Odessa barley (C.I.934), approximately 40 per cent infection resulted on *E. glaucus* but none on the parent hosts. Therefore, there was no indication of segregation of the pathogenicity for either parent.

The F_2 generation of Hybrid 749, as produced on *Elymus glaucus*, consisted of 23 smutted heads. The spore characters of each of these smutted spikes were studied, and it was found that in each one the spores were in-

[3] These hybrid numbers have no other significance than the convenience of using the greenhouse row number in which the F_1 generation occurred.

distinguishable from those of the F_1 generation. They were all distinctly lighter-colored on two sides and were more or less echinulate, especially on the clear sides (Fig. 1). Why there was no segregation in the F_2 genera-

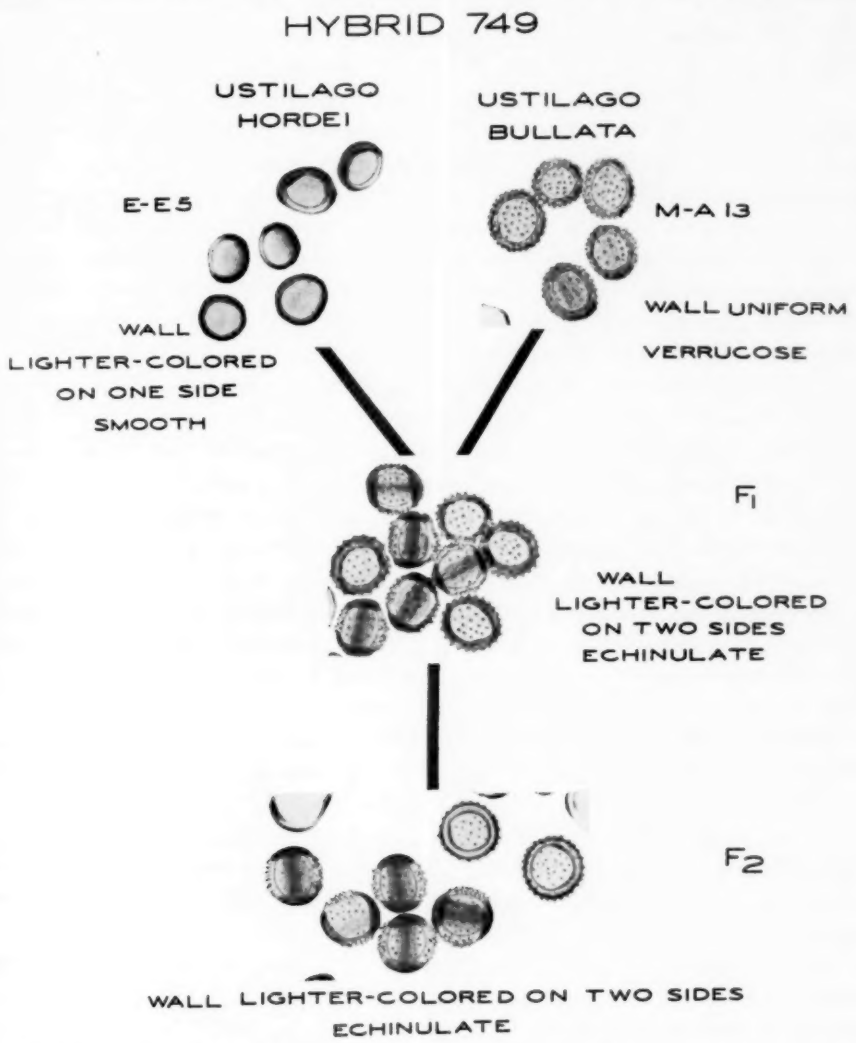

FIG. 1. Hybridization between *Ustilago hordei* and *U. bullata*. Hybrid 749. E–E5 is a collection of *U. hordei* from *Elymus canadensis*. M–A 13 is a collection of *U. bullata* representing Race 2, on cheatgrass, *Bromus tectorum*. Note lack of segregation in the F_2 generation. Retouched photomicrographs.

tion is difficult to explain. Perhaps the one spore type appearing in the F_2 was actually a segregate, the only one finding expression.

Hybrid 751. Hybrid 751 is a cross between the *Elymus canadensis* collection of *Ustilago hordei* and Race 12 of *Ustilago bullata*, as represented by collection M-V, from *Bromus purgans*. The F_1 generation was produced

on *Elymus canadensis*. As with Hybrid 749 the F_1 chlamydospores were neither smooth like the *U. hordei* parent nor verrucose like the *U. bullata* parent, but were echinulate. Furthermore, the spores were lighter-colored on two sides, which is quite in contrast with *U. hordei* and *U. bullata* in which the spores are lighter-colored on one side and uniform in color, respectively.

The F_2 generation was produced on *Elymus canadensis* and *Bromus catharticus*. Only eight smutted heads appeared. A study of the spores of each of these heads revealed segregation into four distinct types: small smooth, somewhat lighter-colored on one side; small echinulate, somewhat lighter-colored on one side; large punctate, with wall thick and uniform in color; and large echinulate, somewhat lighter-colored on one side. The small smooth spores were very similar to those of the *Ustilago hordei* parent. The large punctate spores were the same size as, or somewhat larger than, those of the *U. bullata* parent, had the uniform wall color, but lacked the granular-verrucose exospore. Apparently the small echinulate and the large echinulate types represent additional intermediate segregates.

Of the eight smutted heads comprising the F_2 generation, one contained the small smooth segregate, three contained large punctate, one contained small smooth and large punctate, two contained small echinulate and large punctate spores, and one contained spores representing all four segregates. Three of the eight smutted heads were produced on *Bromus catharticus*, but collectively these contained the same spore types as did those heads produced on *Elymus canadensis*. Inoculations of Odessa barley with F_1 spores gave entirely negative results, thus indicating no segregation of the pathogenicity of the *Ustilago bullata* parent.

Hybrid 752. This hybrid is a cross involving the same collections but different monosporidial lines of both *Ustilago hordei* and *U. bullata* as in Hybrid 749. *Elymus glaucus* served as the common host.

As with Hybrid 749, the F_1 chlamydospores were unlike either parent, being echinulate and lighter-colored on two sides. These spores germinated readily, but monosporidial lines could not be obtained because of extensive lysis of the isolated sporidia.

The F_1 spores were used as inoculum on *Elymus glaucus*, *Bromus tectorum* (the *U. bullata* host), and Odessa barley (the *U. hordei* host). Nine smutted spikes appeared in the *E. glaucus* but no smut developed on the other two hosts. Thus, as with Hybrid 749, there was no indication of segregation of pathogenicity of either parent.

Microscopic examination of the spores from the nine smutted spikes comprising the F_2 generation revealed four distinct types. Three heads contained small smooth and small echinulate spores having a tendency to be lighter-colored on two sides. The other six smutted heads were all different by different combinations of two to four of the four spore types: small smooth, small echinulate, large punctate, and large echinulate. None of these segregating types resembled either parent.

Hybrid 753. For this hybrid, *Elymus glaucus* was used as the common host. The monosporidial lines of both parents were different from those employed in Hybrids 749 and 752, and the segregation was different from segregations obtained in the latter two hybrids.

The F_1 chlamydospores were unlike either parent. As seen in figure 2, they were distinctly lighter-colored and distinctly echinulate on two sides. The dark sides were smooth.

The F_1 spores were used to inoculate *Elymus glaucus, Bromus tectorum,* and Odessa barley. In a single greenhouse row, 23 smutted heads developed on *E. glaucus* but none on the other two. Here again, there was no segregation of pathogenicity of either parent.

When the 23 smutted heads of *Elymus glaucus* comprising the F_2 generation were critically studied microscopically, the echinulate spores of the F_1, lighter-colored on two sides, had segregated into three spore types: lighter-colored on two sides, echinulate; lighter-colored on two sides, smooth; and lighter-colored on one side, echinulate (Fig. 2). Again, none of the segregating types was like either parent.

Sixteen of the 23 smutted heads comprising the F_2 generation of Hybrid 753 contained spores lighter-colored and echinulate on two sides, two contained spores lighter-colored on two sides but smooth, four were lighter-colored on one side and echinulate, and one head contained all three types.

An interesting incidental observation was the striking resemblance of the type lighter-colored and echinulate on one side to the black loose smut of barley and oats, *Ustilago avenae* (Pers.) Rostr. Certain it is that if a smut similar to this particular segregate were found on *Elymus glaucus* in nature, that smut would be determined as *U. avenae.*[4]

Considering the combination of characters in the three segregating types of spores, a fourth might have been found: lighter-colored on one side and smooth. This would have been like the *Ustilago hordei* parent.

Hybrid 754. Using different monosporidial lines of *Ustilago hordei* and *Ustilago bullata* (Race 12) than were used with Hybrid 751, a cross was obtained that behaved somewhat differently from the latter hybrid. This was designated as Hybrid 754. The F_1 was obtained with *Elymus canadensis* as the host common to both parents. The *U. hordei* parent has smooth spores, is lighter-colored on one side, and measures 5–8 μ in diameter. The *U. bullata* parent has granular-verrucose spores, with wall uniform in color, and measures 8–11 μ in diameter.

The F_1 spores (Fig. 3), as with the other hybrids, were intermediate in character between the two parents. They were larger than those of the *Ustilago hordei* parent but smaller than in the *U. bullata* parent. The exospore was echinulate-verruculose, with a scarcely perceptible tendency to be lighter-colored on one side.

Attempts failing to obtain monosporidial cultures from germinating F_1 spores, these were used to inoculate *Elymus canadensis, Bromus catharticus,*

[4] This binomial includes *Ustilago nigra* Tapke (9).

and Odessa barley. Only five smutted heads appeared in a single greenhouse bench row of *E. canadensis* and none in the other two hosts. Here again, there was no indication of segregation of the pathogenicity of either parent.

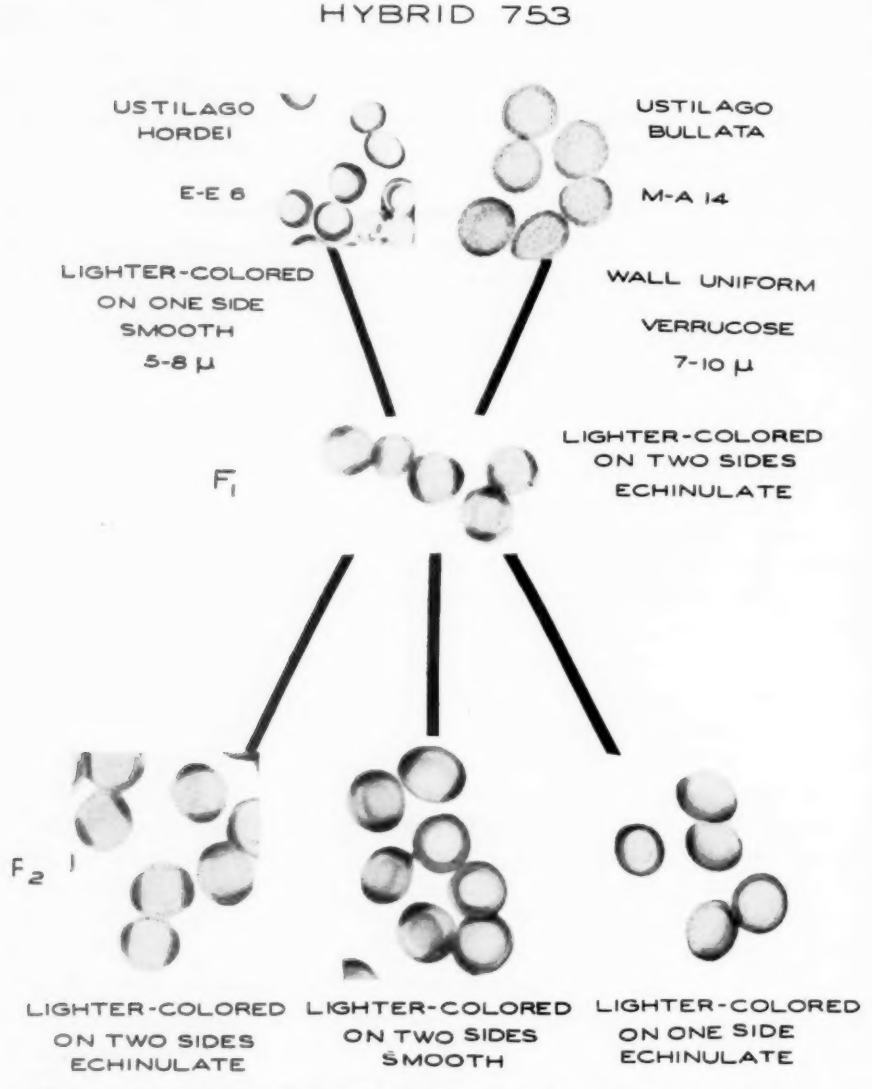

FIG. 2. Hybridization between *Ustilago hordei* and *U. bullata*. Hybrid 753. Same parent races as in Hybrid 749, but from different spores. Retouched photomicrographs.

Four distinct types of segregates were found in the F_2 spores: small (6–8 μ), echinulate; small (5–8 μ), smooth; large (10–14 μ), echinulate; and large (9–11 μ), smooth (Fig. 3). Two of the heads contained small echinulate, small smooth, and large smooth segregates; one contained spores

of the small smooth and large smooth types; one contained large echinulate only; and the fifth contained large echinulate and small echinulate types. The small echinulate and the large echinulate types tended to be lighter-

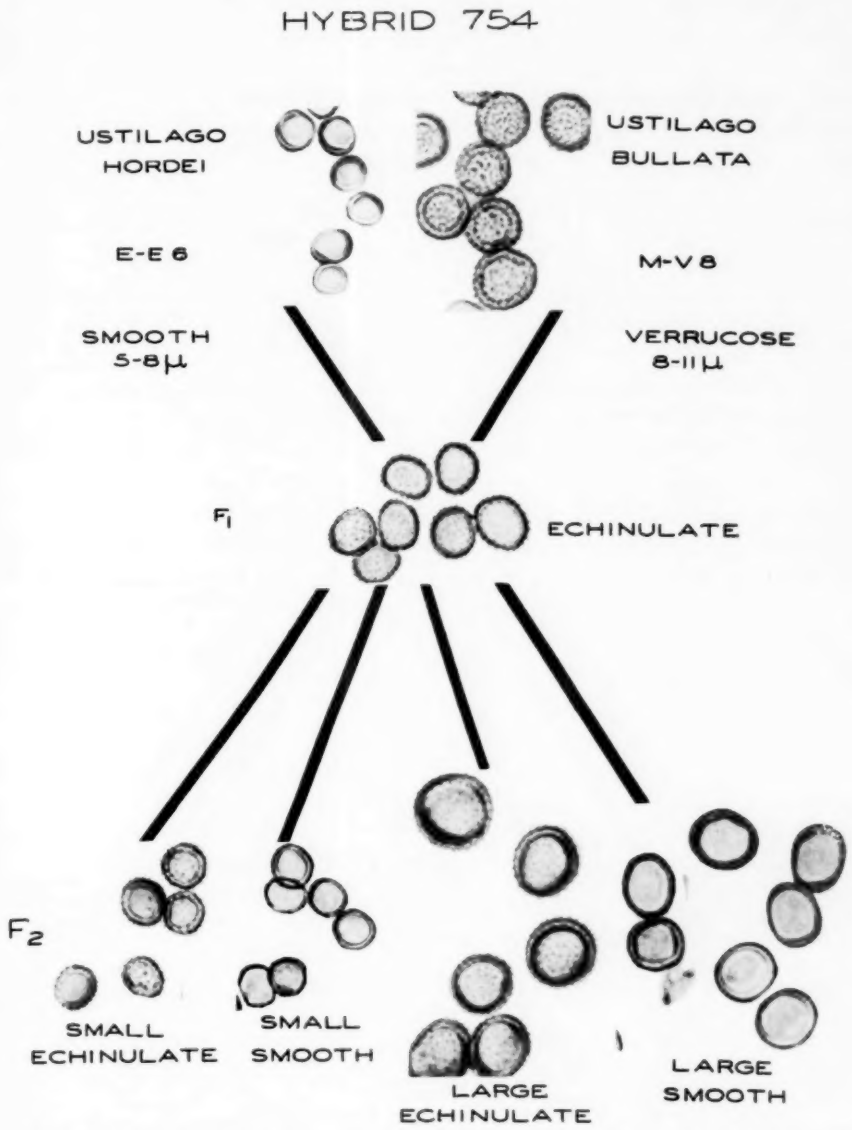

FIG. 3. Hybridization between *Ustilago hordei* and *U. bullata*. Hybrid 754. Same *U. hordei* parent as in Hybrid 753 but the *U. bullata* parent is Race 12 on *Bromus purgans*. Retouched photomicrographs.

colored on one side, with more prominent echinulations on that side. This is apparent only when the spores are oriented to a side view.

None of the F_2 segregates of Hybrid 754 was like either parent. The

small smooth segregate was similar to the *Ustilago hordei* parent except that the wall was uniform in color rather than lighter-colored on one side.

Hybrid 755 is similar to Hybrids 751 and 754, but involved different

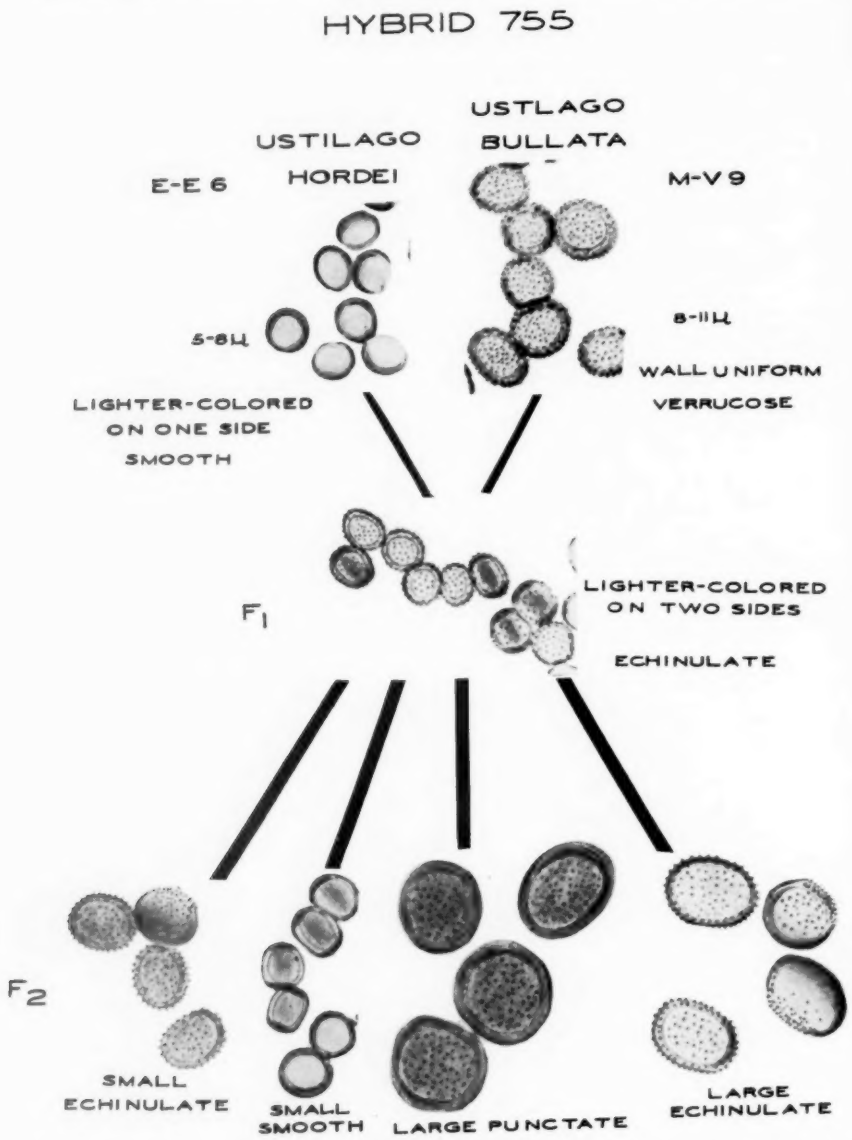

FIG. 4. Hybridization between *Ustilago hordei* and *U. bullata*. Hybrid 755. Same *U. hordei* parent as in Hybrid 753 but the *U. bullata* parent is from a different spore. Retouched photomicrographs.

monosporidial lines. The same contrasting morphologic characters were involved.

The F_1 spores were small, like the *Ustilago hordei* parent, but were

echinulate and lighter-colored on two sides (Fig. 4). They were thus like the spores of the F_1 of Hybrid 751. Inasmuch as monosporidial cultures could not be obtained, the F_1 spores were used as inoculum to obtain the second generation. *Elymus canadensis*, *Bromus catharticus*, and Odessa barley were inoculated. Ten smutted heads appeared in *E. canadensis*, but none in the other two.

In Hybrid 755 the following four spore types segregated in the F_2 generation: small (7–9 μ), lighter-colored and echinulate on one side; small (6–9 μ), smooth, lighter-colored on two sides; large (12–15 μ), punctate; and large (11–13 μ), lighter-colored and echinulate on one side, with the echinulations prominent on that side. The small, echinulate type, like those segregated from Hybrid 753, were very similar to *Ustilago avenae*. The large echinulate spores also closely resembled *U. avenae* except that they far exceeded the size range of this species.

DISCUSSION

The foregoing account of hybridization between *Ustilago hordei* and *U. bullata* shows that in each of the 9 F_1 hybrids the granular-verrucose exospore of *U. bullata* is only partially dominant over the smooth exospore of *U. hordei*, since the F_1 spores were echinulate. This recalls the situation in certain crosses of *Tilletia caries* × *T. foetida*, as reported by Holton (26); the F_1 spores were minutely reticulate in contrast to reticulate and smooth in the two parents, respectively. In other hybrids between the same species, however, smooth exospore was completely dominant over reticulate exospore. Flor (13) and Hanna (15) likewise demonstrated dominance of the smooth character in crosses between *T. caries* and *T. foetida*.

In most studies of hybridization between smooth- and rough-spored smut fungi, however, there has been complete dominance of the latter character. In crosses between *Ustilago avenae* and *U. kolleri*, having echinulate and smooth spores respectively, Hanna and Popp (16), Popp and Hanna (34), and Holton (19, 20) found the echinulate character to be dominant. In hybrids between *Sphacelotheca sorghi* and *S. cruenta*, reported by Rodenhiser (38), echinulation likewise appeared to be dominant. In F_1 hybrids between *Sorosporium syntherismae* and *Sphacelotheca destruens* reported by Martin (33), the spores were verruculose like those of *S. syntherismae* parent.

The indication of partial dominance of the granular-verrucose exospore of the *Ustilago bullata* parent in the present studies unfortuately could not be followed up with definite genetic studies because of low populations in the F_2 generation. *U. hordei* and *U. bullata* are not completely interfertile; all attempts to obtain cultures from the F_1 spores were thwarted by lysis of the promycelia or sporidia.

The role of intra- and, to a lesser extent, interspecific hybridization in the production of new physiologic races of smut fungi has been repeatedly demonstrated (2, 4, 18, 21, 22, 23, 33, 37, 41, 45, 46). Segregates that

might be considered new species have been rare, however. If Martin's (33) F_2 hybrids between *Sorosporium syntherismae* and *Sphacelotheca panici-miliacei*, having echinulate spores in spore balls and smooth spores not in balls, respectively, had yielded segregates having smooth spores adhering in balls or echinulate spores not in balls, these segregates might have been considered new, synthetic "species." Holton's (17) buff smut, considered as resulting from hybridization between *Ustilago avenae* and *U. kolleri*, could have been considered as a new species perhaps, since it did differ from the *U. kolleri* parent in having hyaline spores.

Some of the F_2 segregates in the present studies are suggestive in this regard. The segregates from Hybrids 749, 752, and 753, having spores distinctly lighter-colored and echinulate on two sides, might be considered as a new species (depending upon one's species concept!). There is no such smut known to the writer, occurring on *Elymus* spp., although there is one on *Muhlenbergia* spp.[5] Similarly, the segregates from Hybrids 753 and 755, having smooth spores, lighter-colored on two sides, certainly are not like either parent and are different from any described species of *Ustilago* on grasses. The same might be said of the large, smooth-spored segregate in Hybrid 754.

The segregates in Hybrids 751, 753, 754, and 755 having spores lighter-colored and echinulate on one side strongly suggest the appearance of *Ustilago avenae* (*U. nigra*, *U. perennans*). Some were outside the size range of *U. avenae* but the others were scarcely if at all distinguishable from this species. It would be trite to attempt to elaborate on the origin of species by hybridization, but the segregates described above do provide further suggestive evidence of the phenomenon, especially as pertaining to the smut fungi.

Although the F_1 spores were more or less intermediate in size, those of some of the F_2 segregates were considerably larger than is characteristic of either parent. This may be a manifestation of heterosis.

SUMMARY

An account is given of hybridization between one race of *Ustilago hordei* (spontaneously occurring on *Elymus canadensis*) and three races of *U. bullata*.

Attempts at hybridization were based on compatible reactions between pedigreed monosporidial cultures of the two species. Fourteen such compatible combinations were used to inoculate seedlings of *Elymus canadensis* and *E. glaucus*, two hosts selected as common to both *U. hordei* and *U. bullata*.

Several contrasting morphological characters were available for study in crossing these smut species: *Ustilago hordei* has small (5–8 μ), smooth spores, lighter-colored on one side; *U. bullata* has larger (8–12 μ), granular-verruculose spores, with wall uniformly dark.

[5] *Ustilago hyalino-bipolaris* G. W. Fisch. & Hirschh. Mycologia 37: 318–325. 1945.

Nine hybrids were obtained. In each case the F_1 spores were echinulate, and lighter-colored on two sides. Spore germination was easily obtained, but all attempts to obtain monosporidial cultures were unsuccessful because of extensive lysis of promycelia and sporidia. Therefore, spores were used as inoculum to obtain F_2 sori.

Six of the nine F_1 hybrids yielded a second generation. Three involved Race 2 of *Ustilago bullata* (common on cheatgrass, *Bromus tectorum* west of the Rocky Mountains), and three Race 12 (on other *Bromus* spp.).

In one of the hybrids, the second-generation spores were all of one type, and exactly like those of the F_1, that is, lighter-colored and echinulate on two sides.

In the F_2 generations of the other five hybrids, segregation resulted in three or four of the following spore types: small (5–8 μ), echinulate, lighter-colored and more prominently echinulate on the lighter side; small (5–8 μ), smooth, lighter-colored on two sides; same, but lighter-colored on one side (i.e., like the *U. hordei* parent); small (5–8 μ), lighter-colored and echinulate on one side; same, but large (9–13 μ); large (11–15 μ), punctate, with uniformly colored wall; and large (9–11 μ), smooth, with uniformly colored wall. The spores of the large types, being in some cases several microns larger than the larger (*U. bullata*) parent, suggest heterosis. The populations in the F_2 generation were too small to permit ratio studies.

The small, echinulate segregates, lighter-colored on one side, are very similar to *U. avenae*. Some of the other segregates appear to be distinct from established species on grasses of the genera concerned. The theory of hybrid origin of species of smut fungi is considered to be further substantiated.

In only one hybrid was there segregation of pathogenicity of the *U. bullata* parent. In all the others, the F_1 spores failed to infect the hosts of either parent and thus thwarted attempts to observe inheritance of pathogenicity.

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PHYTOPATHOLOGICAL NOTES

Soreshin of Cotton and Its Relationship to Thrips Damage. D. C. NEAL AND L. D. NEWSOM. Soreshin of cotton, first described by Atkinson in Alabama in 1892 and 1896[1, 2] and caused by the fungus *Rhizoctonia solani* Kühn, is probably the most destructive seedling disease of this crop. It is frequently responsible for loss of stands to such an extent that replanting becomes necessary. No satisfactory control measure is known for the disease.

In studies of control of the tobacco thrips, *Frankliniella fusca* (Hinds), conducted in the Red River Valley area of Louisiana during 1950, it was observed that in the plots where thrips were controlled fewer of the plants were affected by soreshin. Because of the marked contrast in the prevalence of the disease between plants of the treated and untreated plots on casual examination, the soreshin-affected plants were counted on June 16. Of 200 plants collected at random from the treated plot, 153 were healthy, 30 had mild soreshin infection, 13 were moderately infected, and four plants had severe soreshin. Of another 200 plants collected from an adjoining untreated plot, only 30 were healthy, 51 had mild infection, 44 had moderate infection, and 75 were severely infected with soreshin.

When seedling cotton plants are damaged by thrips the leaf area is greatly reduced. In these experiments the leaves were blueprinted and the actual area was determined by means of a polar planimeter. The plants from the treated plots had 70 per cent more leaf area than those from the untreated plots. This reduction in leaf area as the result of thrips injury suggests that the photosynthetic activity and the translocation of elaborated food are probably also greatly reduced, resulting in a weakened plant much more susceptible to soreshin attack.—Division of Cotton and Other Fiber Crops and Diseases, U. S. Department of Agriculture, and Department of Entomology, Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana.

Spinach Smut, Entyloma ellisii Halsted, a New Record for Washington State.[1] WILLIAM D. YERKES, JR., CHARLES GARDNER SHAW, AND GEORGE W. FISCHER. During routine examination of the foliage of *Spinacia oleracea* L. for oospores of *Peronospora farinosa* (Fr.) Fr., spores suggestive of an *Entyloma* were found in mildew-infected leaves collected at Walla Walla, Washington, on April 17, 1950, by the two senior authors. The available published references to a smut of spinach were based on the original record of *Entyloma ellisii* as described in 1890 by Halsted who found the smut in spinach hot beds in New Jersey: the spores were formed in

[1] Atkinson, G. F. Some diseases of cotton. Ala. Agr. Exp. Sta. Bul. 41. 1892.
[2] Atkinson, G. F. Diseases of cotton. U. S. Dept. Agr. Off. Exp. Sta. Bul. 33: 279–316. 1896.
[1] Scientific Paper No. 1010, Washington Agricultural Experiment Stations, Institute of Agricultural Sciences, The State College of Washington, Pullman.

"pale white, indefinitely limited, subconfluent" spots, and were nearly colorless, spherical, chiefly 16–20 μ in diameter, and thick-walled (2–5 μ).[2]

A portion of Halsted's type material, loaned through the courtesy of J. A. Stevenson, was examined. The leaf symptoms described by Halsted were not confirmed because of discoloration of the spinach leaves with age. Spores in this specimen, very sparsely present, measure 16–28 μ, and possess thick gelatinous walls which vary from 2 to 7 μ in thickness (Fig. 1, C). Obviously Halsted included in his spore measurements the gelatinous material composing the spore wall.

Although Halsted described conidia,[2] none were found in the portion of the type available to us. Nor have conidia yet been seen in material from Washington State.

The spores first observed in the Washington State material were in irregular, marginal, necrotic lesions (4–6 mm.). The spores measured but 10–14 μ in diameter. They lacked the thick gelatinous sheaths of the spores in Halsted's material, the wall measuring but 0.5–0.8 μ (Fig. 1, A). Further examination of this material, however, disclosed spores resembling

TABLE 1.—*Mean spore and spore-wall measurements (in microns) of Entyloma ellisii*

Source	Spore wall	Protoplasmic content	Total diameter
Halsted's Type material			
25 spores	3.5	15.1	22.1
Washington State material			
50 immature spores	1.9	15.5	19.3
50 mature spores	0.6	10.6	11.8

more closely those in Halsted's material. These were in minute, intercostal, faintly chlorotic lesions (1–2 mm.), measured 15–34 μ in diameter, and possessed gelatinous spore walls 1.3–2.0 μ thick (Fig. 1, B).

The differences among the spore measurements obtained from these two specimens may be explained partially, at least, on the basis of degree of maturity. The hyaline spores with gelatinous sheaths are considered immature, the sheath being thinner on the more mature pale brown spores. That these spores are found in chlorotic lesions strengthens this hypothesis. The relatively thin-walled pale brown spores in necrotic lesions in the Washington State material are considered mature. Average measurements (Table 1) disclose that 1) the immature spores in the Washington State material agree fairly well with the spores in the Halsted material, the major difference being in the thickness of the wall; and 2) there is considerably greater variation between the mature and immature spores in the Washington State specimen than there is between the immature spores of that collection and the spores of Halsted's type material.

The Washington State record of *Entyloma ellisii* arouses speculation concerning the occurrence of the smut during the past 60 years and in

[2] Halsted, Byron D. A new white smut. Bull. Torr. Bot. Club 17: 95–97. 1890.

other spinach-growing areas between New Jersey and Washington State. The indistinct nature of the lesions is most likely responsible for its having escaped notice. We certainly did not see the smut lesions at the time our specimen was collected. Indeed, the lesions were not noted until after spores had been found under the microscope. It is also interesting to note

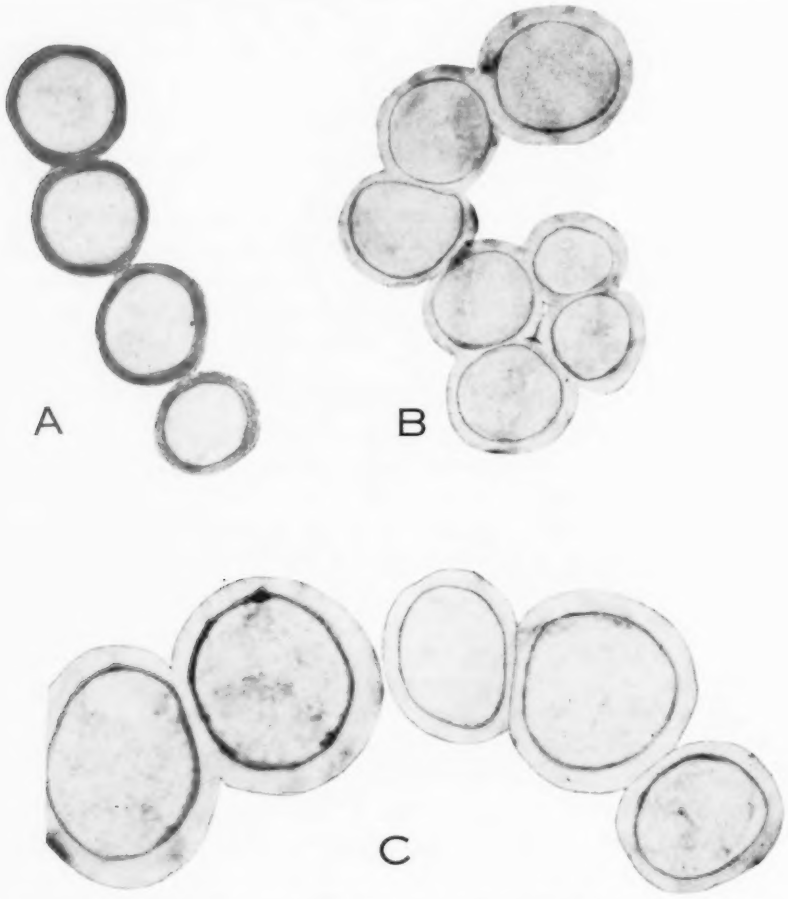

FIG. 1. *Entyloma ellisii*. A and B. Spores from Washington State collection: A, mature; B, immature. C. Spores (immature?) from type material of the Halsted collection from New Jersey. Retouched photomicrographs, × 1150.

that our collection was made in a field of spinach, whereas the original collection was discovered in a seed bed. The smut's presence in the field also suggests that it may have been present in trace amounts from year to year but overlooked.—Department of Plant Pathology, State College of Washington, Pullman, Washington.

A Glass-Tumbler–Paper-Doll Technique for Seed Corn Incubation and Germination Tests. PAUL E. HOPPE.[1] A modification of the glass-tumbler

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method[2] permits extensive controlled germination and disease tests of seed corn in cold soils without either large refrigeration equipment or greenhouse facilities. In the glass-tumbler–greenhouse method corn kernels are embedded in soil in glass tumblers, incubated at a low temperature in an ordinary electric refrigerator for about 10 days, and then removed from the tumblers and planted in a warm greenhouse. Data are recorded when healthy seedlings reach the 3- to 4-leaf stage.

The new technique eliminates the need for a greenhouse. Kernels removed from the tumblers are placed on moist paper towels which are rolled

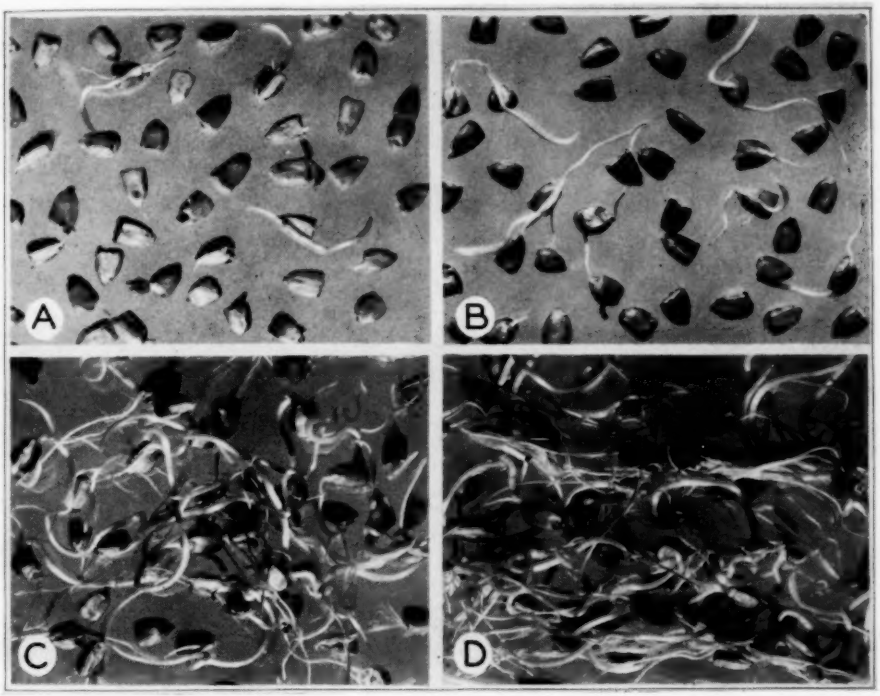

FIG. 1. Germination of crown-wounded corn kernels. (A) Untreated and treated with (B) Spergon, (C) Arasan, and (D) Experimental Fungicide No. 224, by the glass-tumbler–paper-doll technique. Results agree closely with those obtained by former methods of testing.

into "rag dolls" and put in covered metal pans at room temperature. Germinations are recorded about 4 days later. The glass-tumbler–paper-doll method apparently can be used for almost any corn seedling disease tests in cold soils. It is satisfactory for measuring: 1) efficiency of different dust seed treatments (Fig. 1), 2) toxicity of fungicides at different dosages, 3) differences in resistance to disease among inbred lines of corn, and 4) effect of rough handling of seed upon disease development.

The method is simple, reliable, and much faster than those used heretofore. Since the only special equipment needed is an ordinary refrigerator,

[2] Hoppe, Paul E. A new technique for incubating seed corn in cold soil for disease tests. (Abstr.) Phytopath. 41: 18. 1951.

the new technique should find wide usage in seed testing and phytopathological laboratories. It should prove particularly useful to manufacturers of fungicides for rapid screening of experimental fungicides, and for special tests with the more promising products.—U. S. Department of Agriculture, and University of Wisconsin, Madison, Wisconsin.

Control of Strawberry Red Stele by Chemotherapy. E. M. STODDARD.[1] The red stele disease of strawberry, caused by *Phytophthora fragariae,* has been controlled by soil applications of disodium ethylene bisdithiocarbamate (Dithane D14). There is evidence that the material was taken up by the roots and that it acted as a therapeutant.

In a greenhouse experiment, two units of 10 Fairpeake strawberry plants potted in sand were watered with 25 ml. of water solution of Dithane D14 per pot on five successive days, at concentrations of 1.5 and 0.75 parts to 100 parts of water, respectively. After the treatments the plants were removed from the sand and the roots thoroughly washed in tap water and set in infested soil. The plants were grown for 2 months in the infested soil at 7° C. without further treatment. At this time the plants receiving the stronger concentration had 0.00 per cent disease, and those with the weaker concentration, 40 per cent disease. A similar unit of check plants had 90 per cent disease. This indicates that the Dithane D14 was absorbed by the plants and acted as a therapeutant and not as a soil sterilant.

Two units of like plants were potted in infested soil and treated with the same concentrations of Dithane D14 described above. Two months after the treatment with 1.5 and 0.75 concentrations, 30 per cent and 0.00 per cent, respectively, of the plants were diseased, as compared to 90 per cent in the checks. It is thought that root injury from the higher concentration may have reduced uptake of the material with consequent reduced control.

These results indicated the probability of successful field control of red stele of strawberry and accordingly the following field trial was undertaken.

Dithane D14 at a concentration of 1.5 parts per 100 parts of water was injected under pressure into the soil in an infected area. The entire infected area was treated, as well as a band 10 ft. wide on the perimeter of the area. The time was early May as the plants were starting to bloom, and the rate of application was approximately 2000 gal. per acre. The result of the treatment was immediate stopping of further spread of the disease and no injury to healthy plants in the adjacent treated band. A second treatment was made after picking. No spread of the disease occurred in the same or the following year and healthy plants set in the treated infested area remained healthy.

It seems probable that in the field experiment Dithane D14 was acting both as a therapeutant and as a soil sterilant.—Connecticut Agricultural Experiment Station, New Haven, Connecticut.

[1] The writer is indebted to his assistant, Frances W. Meyer, for suggestions and assistance in conducting the work, and to Dr. George Darrow, U. S. Department of Agriculture, Beltsville, Maryland, for the technique of growing the plants to insure infection.